

LABORATORY MANUAL

BY

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The Development, Organization and Operation of the Bureau of Laboratories

of the

Michigan Department of Health

C. C. Young, Director

A dissertation submitted in partial fulfillment
of the requirements for the degree of Doctor
of Public Health in the University of Michigan

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MICHIGAN DEPARTMENT OF HEALTH
 Bureau of Laboratories.
COOPERATE !!
 Use Our Containers.
 Sent on Request.

The 'M' shape is composed of the following labels and images (from top to bottom, left to right):

- Top Left:** Blood for Culture, Blood for Typing, Blood for Serology, Blood for Wassermann, Blood for Treponema, Blood for Syphilis, Blood for Rheumatism, Blood for Glandular Disease, Blood for Kidney Disease, Blood for Liver Disease, Blood for Heart Disease, Blood for Lung Disease, Blood for Stomach Disease, Blood for Intestine Disease, Blood for Genital Disease, Blood for Skin Disease, Blood for Nervous System Disease, Blood for Endocrine System Disease, Blood for Reproductive System Disease, Blood for Immune System Disease, Blood for Circulatory System Disease, Blood for Respiratory System Disease, Blood for Digestive System Disease, Blood for Urinary System Disease, Blood for Musculoskeletal System Disease, Blood for Integumentary System Disease, Blood for Sensory System Disease, Blood for Motor System Disease, Blood for Central Nervous System Disease, Blood for Peripheral Nervous System Disease, Blood for Autonomic Nervous System Disease, Blood for Endocrine System Disease, Blood for Reproductive System Disease, Blood for Immune System Disease, Blood for Circulatory System Disease, Blood for Respiratory System Disease, Blood for Digestive System Disease, Blood for Urinary System Disease, Blood for Musculoskeletal System Disease, Blood for Integumentary System Disease, Blood for Sensory System Disease, Blood for Motor System Disease, Blood for Central Nervous System Disease, Blood for Peripheral Nervous System Disease, Blood for Autonomic Nervous System Disease.
- Top Right:** Blood for Culture, Blood for Typing, Blood for Serology, Blood for Wassermann, Blood for Treponema, Blood for Syphilis, Blood for Rheumatism, Blood for Glandular Disease, Blood for Kidney Disease, Blood for Liver Disease, Blood for Heart Disease, Blood for Lung Disease, Blood for Stomach Disease, Blood for Intestine Disease, Blood for Genital Disease, Blood for Skin Disease, Blood for Nervous System Disease, Blood for Endocrine System Disease, Blood for Reproductive System Disease, Blood for Immune System Disease, Blood for Circulatory System Disease, Blood for Respiratory System Disease, Blood for Digestive System Disease, Blood for Urinary System Disease, Blood for Musculoskeletal System Disease, Blood for Integumentary System Disease, Blood for Sensory System Disease, Blood for Motor System Disease, Blood for Central Nervous System Disease, Blood for Peripheral Nervous System Disease, Blood for Autonomic Nervous System Disease.
- Left Side (Vertical):** Sputum, Feces, Urine Bacteriological, Urine.
- Right Side (Vertical):** Sputum, Feces, Urine Bacteriological, Urine.
- Center (Vertical):** Throat Swab, Pus Smear for Gonococcus Examination, Blood Smear for Differential Count, Veterinary Examinations.
- Bottom:** Urine Chemical.

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PREFACE

This dissertation may best be considered as a five year general report on the laboratories of the Michigan Department of Health and a description of both technical and administrative procedure now in use. It is hoped that this record of the experience of building up a going concern will help other laboratory directors to render decisions between several courses of procedure. The author makes no claim to originality. He has applied in the Michigan Department of Health the ideas gleaned from visits to other laboratories, has used material which appeared in technical journals from time to time, but in most cases the schemes have been worked out by trial and error. This report, therefore, is the epitome of fifteen years of experience in directing laboratories.

The criterion for adopting any procedure is maximum service at the least cost; the policy, accuracy and dependability of results at any cost. The writer believes that the laboratory is a compact, efficient organization in good standing with the medical profession and giving a maximum of service to the taxpayer.

ACKNOWLEDGMENTS

Without the broad sociological vision of Dr. R. M. Olin, Commissioner of Health, the work of the building up of the Michigan Department of Health laboratories would have been impossible. His judgment of men and the perception of the psychological moment for presentation of a problem to other state executives and to the medical profession have made him one of the foremost health executives in America. He has correlated the work of the seven bureaus so that all of them have had almost equal development. He has at every turn set the pace for the bureau directors to follow.

Special recognition is due: Miss Crooks for her untiring interest in the minutest details of procedure in the Division of Microbiology, and for her clear-cut executive decisions as assistant to the director; Dr. R. L. Kahn for putting the serological diagnosis of syphilis upon a dependable basis; Miss Kendrick for the development of the Media Division; Mr. Bliss for relieving the director of any responsibility for medical legal work; and Miss Ora Mills for her work in the Upper Peninsula laboratory.

The cooperation and loyalty of Mrs. Juliette Bartholomew Stucky, executive secretary of the laboratory, and Mrs. Frances Strang Cleland, secretary to the director, are gratefully acknowledged.

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PART I

**THE DEVELOPMENT AND
ORGANIZATION**

CHAPTER I

THE BEGINNING OF LABORATORY SERVICE TO PHYSICIANS IN MICHIGAN

Some time prior to 1897 the Hygienic Laboratory was established by the regents of the University of Michigan, as indicated by Act 43, Public Acts of 1897, and some time prior to 1903 the Pasteur Institute was established, as indicated by Act 116 of the Public Acts of 1903. The exact dates we have been unable to secure either from the Compiled Laws of the State of Michigan or by inquiry at the university. Apparently the Hygienic Laboratory largely concerned itself with the examination of water and certain research problems. The Pasteur Institute was empowered only to manufacture rabies virus and give treatment for rabies to indigents.

It was not until 1907 that any attempt was made to furnish diagnostic aid to physicians in Michigan. The Legislature of 1907 passed Act 109 which provided for the appointment of a bacteriologist by the State Board of Health and for the necessary appliances and apparatus for bacteriological examinations. The law provided for the use of this laboratory by various boards of health, health officers and state institutions which might require examinations or analyses of blood, sputum, urine, water and milk, or other substances in case of outbreaks of contagious diseases or epidemics in which bacteriological examination or analysis might be necessary to the public health and welfare. The act also provided for chemical examinations of criminal nature which might be required by prosecuting attorneys. It further established a fee system to be used at the discretion of the Board for the maintenance of the laboratory.

In the Public Acts of 1915, Act 164 provided for the establishment of a branch bacteriological laboratory in the upper peninsula, locating the laboratory at Houghton. The Act had many of the provisions of the Act of 1907 for the Lansing laboratory. The Lansing laboratory operated for seven years before the Houghton branch was established, so that the type of work done in the Houghton laboratory was largely patterned after the Lansing ideas. These two laboratories had no interrelationship whatever. They reported separately to the Secretary of the State Board of Health and had separate funds for maintenance. Up to 1916 the work covered by these two laboratories was limited to the following endeavors: Post-operative pathology, toxicology, diphtheria diagnosis, Widal tests, stains for gonococcus, sputum for tubercle bacilli, bacteriological examinations of milk and water, qualitative urine analysis for insurance companies, and an occasional differential blood count. There was a fee system in vogue for post-operative pathology and any special work that might be desired by physicians or individuals.

In 1916 the Wassermann test was begun in the Lansing laboratory and put on a fee basis. It showed no great development until July, 1917, when put

on the free list. On account of the wartime emphasis upon venereal diseases, the Wassermann test was then put on the free list. The Board of Auditors authorized this procedure, provided a slip was sent to each patient stating that the test was made free of charge by the state and that the report could be obtained from the doctor. This ruling is still in effect and is handled by making a carbon copy of that portion of the Wassermann report that carries the name and address of the physician and the name and address of the patient. The merits or demerits of this procedure are not a matter for discussion in this report, as it is a departmental administrative measure which has a great many virtues and some drawbacks. The real beginning, then, of laboratory service, the writer believes, started with the placing of the Wassermann test on the free list in July, 1917. The fundamental principle of charging for diagnostic tests will be discussed later in another chapter.

Abolition of the State Board of Health and the Formation of the Michigan Department of Health

The Legislature of 1919 passed Act 146 which is "An act to protect the public health, to provide for the appointment of a state health commissioner, deputy state health commissioner, and state advisory council of health; to provide the compensation, powers and duties thereof, the powers and duties of township, village and city health officers and health boards; and to abolish the state board of health." The then Secretary of the State Board of Health, Dr. R. M. Olin, was appointed the first Commissioner of Health by Governor

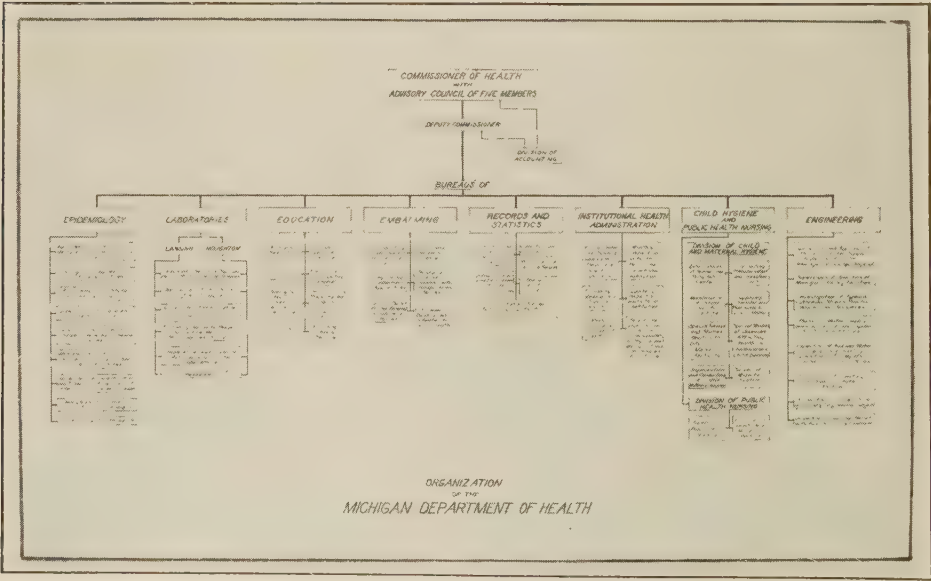


Chart I

Sleeper. The Michigan Department of Health was organized by the commissioner under seven bureaus as illustrated in Chart I. As indicated, the Houghton laboratory and the Lansing laboratory were joined into the Bureau of Laboratories of the Michigan Department of Health. On discharge from the army early in 1919, the writer was appointed as director of laboratories of the Michigan Department of Health.

It is worthy of note, in the light of oldtime practice in public health affairs, that the agreement with the commissioner for tenure of office was based entirely upon the results accomplished, or in other words, as long as the director of laboratories was compatible with the rest of the members of the staff and was delivering satisfactory service to the physicians of Michigan and to the other bureaus, he could hold office. The instructions from the commissioner were about as follows: "Run the technical side of your laboratory to get the best results possible, and refer to the commissioner only administrative matters which bear upon the relation of the department of health to the public or the relationship of the bureau of laboratories to other bureaus or state departments." With this beginning the status of the bureau of laboratories was defined as a subsidiary bureau which should function as a scientific aid to all other bureaus of the state department of health. It should serve the public through the physicians in a perfectly ethical manner with just as much regard for the physician and his patient as if the physician were doing the work himself. It should institute research in the biological sciences as well as do the technical routine tasks.

After a study of the laboratory records and correspondence of several years, the director of laboratories made recommendations which were approved by the commissioner and formed the basis of laboratory policy. These include the elimination of post-operative pathology, the immediate extension of cultural bacteriology, reorganization of the Wassermann test, establishment of controls and checks upon the routine microscopy, the discontinuance of all fees except where the examinations should be made by the physician to be of maximum value or where tests bore absolutely no relation to possible source of infection.

The reasons for these various changes and additions to the service were based upon a belief that the trend of preventive medicine was in the direction of life extension rather than in the study of sources and modes of infection. Laboratories should not only do all the necessary tasks to control quarantine and aid in the diagnosis of infections, but should be prepared and ready to extend their service to a point where they would be an aid to physicians in the routine examination of patients who wish to keep well. Cost of the necessary laboratory service which accompanies a thorough physical examination makes a systematic annual overhauling an impossibility for 90 per cent of the people. It is therefore within the realm of the fundamental ideals of public health work in Michigan that the state should undertake

these technical tests which lie in the twilight zone between biological science and the practice of medicine.

Post-operative pathology was discontinued for the reason that more than 90 per cent of the cases on record in the department involved a question of tumor diagnosis or a question of surgical judgment which had come into controversy. These questions between patient and doctor were considered to be civil matters and of no interest to the department of health.

The Wassermann test, as run by the department, had too few controls and, although doing fairly satisfactory work, did not meet the specifications that the commissioner had laid down in his statement that the work of the laboratory should be of the very highest order.

When the fee system was overhauled, all fees were abolished except a charge for qualitative urine analysis for albumin and sugar and for toxicological examinations done for prosecuting attorneys. In the light of the development of child hygiene and care for expectant mothers,* in all probability the fee for urinalysis will be abolished except for insurance companies.

By the end of 1919 the laboratories of the Michigan Department of Health were prepared to make practically all of the approximately 150 examinations that would be called for in any diagnostic laboratory.

CHAPTER II

THE GROWTH OF THE LABORATORY FROM 1919 to 1923

While it was expected that the abolition of the fee system and the increasing of the variety of work would stimulate the use of the laboratory, not in his most sanguine moments did the director of laboratories expect such a steady, progressive increase in the volume of work as has been experienced from the summer of 1919 to the present date. An eight hundred per cent increase in four years brought out administrative problems that could only be met by the most whole-hearted cooperation from the Commissioner, the Governor, the Board of Auditors and the State Administrative Board. It was necessary time and again to call upon the general funds of the state to meet this enormous demand for laboratory service. The budget which was made out in the fall of 1918 and passed by the Legislature of 1919 for the two years, 1919 and 1920, was based upon the experience of ten years. When making out the budget in the fall of 1920, basing it upon the experience of 1919-1920 and adding what was thought to be a reasonable amount for increase, it was found that the funds again fell short. When the budget went to the Legislature of 1923, it was based upon the progressive growth and the flattening out of the curve for 1921, 1922 and 1923. (See Chart II.) It was assumed that the service had about reached the peak load until the population of the

* F. F. Russell, M. D., *New Fields of Usefulness for the Public Health Laboratory*, *Am. J. of Pub. Health*, XIII: 995. (Dec.) 1923.

state increased, but there has been a thirty per cent increase for the first six months of the fiscal year 1923-1924, so that there are financial shoals ahead.

Careful check upon each month's business is necessary to analyze the progress of development. Certain progress reports have been required to keep a constant watch upon the changes in the demand for each type of service. A record book of examinations, in which is kept a daily analysis of our business, indicates the number and result of each type of examination.

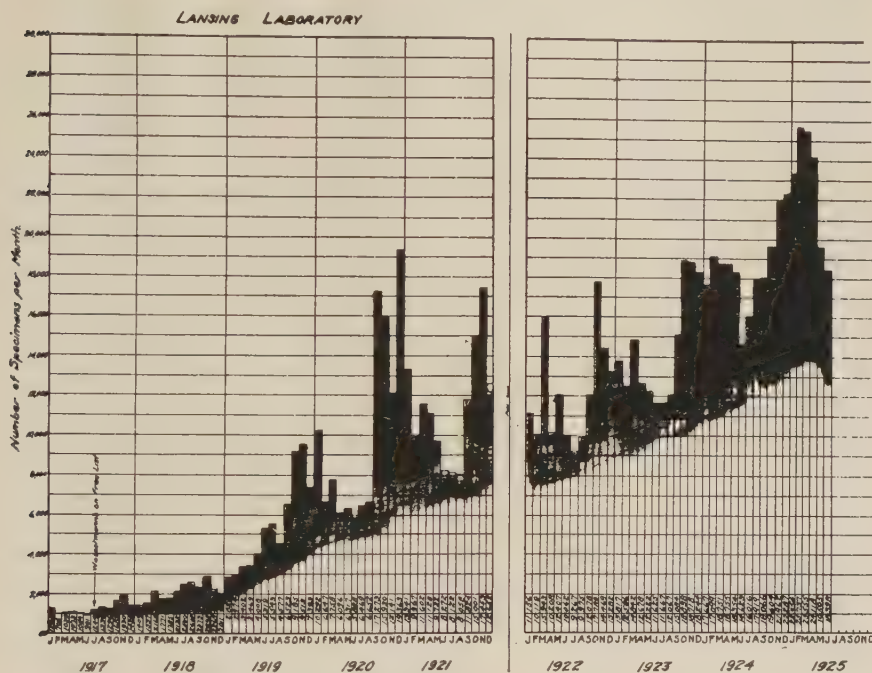


Chart II

This record is posted one day behind the outgoing reports and serves not only as a check upon the volume of the day's business but serves also as an inspection of the outgoing reports. As the reports for each day are counted, they are checked off from the mail entry blank. (Appendix F.) Consequently no specimen can be lost in the laboratory. The daily totals of the record book are checked back against the same day of the same month of the year previous. The increases or decreases for the day and the total increases or decreases for the month are entered in the two right hand columns. On the first day of each month, the preceding month's business is balanced in the record book and the totals constitute the monthly report to the commissioner. The second page of the monthly report carries the industrial report for the month, that is, the number of outfits mailed, the amount of media produced, the amount of antitoxin distributed, the number of laboratory animals used and the cost of operation for that month.

TABLE 1—MICHIGAN DEPARTMENT OF HEALTH—BUREAU OF LABORATORIES
MONTHLY REPORT JUNE, 1923
LANSING LABORATORY

	Number.	This month last year.		Since July 1.
		Inc.	Dec.	
THROAT SWABS FOR DIAGNOSIS.....	1,587	637		21,219
Diphtheria +.....	101			1,508
Diphtheria -.....	389			5,766
H. Strep +.....	142			2,356
Green Strep +.....	261			2,379
Vincent's +23-452.....	475			6,470
Strep -.....	219			2,740
THROAT SWABS FOR RELEASE.....	396	40		6,882
Diphtheria +.....	132			2,352
Diphtheria -.....	264			4,476
H. Strep +.....				31
Strep -.....				23
THROAT SWABS FOR CARRIER.....	430		833	20,601
Diphtheria +.....	41			473
Diphtheria -.....	249			12,971
H. Strep +.....	17			3,681
Strep -.....	123			3,476
VIRULENCE TESTS, DIPHTHERIA.....	14			251
Positive.....	12			186
Negative.....	2			65
WASSERMANN REACTION.....	3,078	707		34,101
Positive.....	651			7,122
Negative.....	2,359			25,963
Doubtful Pos.....	68			1,016
Spinal Fluid.....	(18)			(278)
KAHN PRECIPITATION TEST.....	2,916	646		32,166
Positive.....	687			6,997
Negative.....	2,184			24,239
Doubtful Pos.....	45			930
EXAMINATIONS FOR GONOCOCCI.....	1,249	173		12,798
Smears.....	1,191			12,570
Positive.....	150			1,827
Negative.....	1,041			10,743
Culture + -4.....	4			10
Comp. Fix. +2-52.....	54	569	76	218
SPUTUM EXAMINATIONS.....				5,238
B. Tuberculosis.....	534			5,019
Positive.....	67			723
Negative.....	467			4,296
Miscellaneous.....	32			175
Animal Inoc.....	3			44
TRANSUDATES AND EXUDATES.....	113	31		1,097
Bacteriological.....	64			678
Chemical.....	39			324
Animal Inoc.....	10			94
Serological.....				1
BLOOD EXAMINATIONS.....	183	81		1,975
Blood Cultures.....	13			236
Chemical Analyses.....	58			555
Blood Counts.....	56			431
Blood Groupings.....				4
Widal.....	56			749
Positive.....	4			207
Negative.....	51			519
Doubtful.....	1			23
FECES EXAMINATIONS.....	89		3	2,147
B. Typhosus.....				
Diagnosis +1-49.....	50			1,333
Release +.....				
Parasites.....	26			706
Miscellaneous.....	13			108
URINE EXAMINATIONS.....	293	76		3,817
Sediment.....	117			1,509
Bacteriological.....	26			582
Chemical.....	134			1,630
Animal Inoc.....	16			99
WATER AND SEWAGE EXAMINATIONS.....	231		38	2,928
Bacteriological.....	224			2,828
Chemical.....	7			100
MILK EXAMINATIONS.....	76	76	13	894
VETERINARY EXAMINATIONS.....	43		37	584
TOXICOLOGICAL EXAMINATIONS.....	1		10	46
MISCELLANEOUS EXAMINATIONS.....	18	14		1,792
AUTOGENOUS VACCINES.....	7	1		60
SUPPLEMENTARY EXAMINATIONS.....	332	15		6,034
TOTAL EXAMINATIONS.....	11,625	1,563		154,630

TABLE 2—MICHIGAN DEPARTMENT OF HEALTH—BUREAU OF LABORATORIES
MONTHLY REPORT JUNE, 1923
LANSING LABORATORY

	Number.	This month last year.		Since July 1.
		Inc.	Dec.	
OUTFITS MAILED:				
Swabs for Cultures.....	834		920	40,915
Vials for Blood.....	4,076		1,304	52,144
Jars for Sputum and Feces.....	809		214	11,712
Glass Slides.....	208		613	5,397
Metal Slides.....	31	1		618
Bottles for Water and Urine.....	357	28		4,340
MEDIA MANUFACTURED:				
Tube Media (Plain).....	11,600	1,550		134,900
Tube Media (Special).....	5,975		1,725	105,540
Plate Media (Plain).....	7,850	1,850		79,105
Plate Media (Special).....	1,292	142		31,799
ANTIGEN.....	500 c. c.			4,150 c. c.
AMBOCEPTOR.....				210 c. c.
DIAGNOSTIC SERA.....				947 c. c.
TYPHOID VACCINE.....				
MEDIA SOLD				
NUMBER UNITS DIPHTHERIA ANTITOXIN DISTRIBUTED.....	8,243,000			302,127,000
ANIMALS USED:				
Rabbits.....	15	7		152
Guinea Pigs.....	125	26		1,799
USERS:				
New Physicians.....	12		7	284
New Health Officers.....				1
Typhoid Vaccine Manufactured.....				16,634 c. c.
Typhoid Vaccine Distributed (old), 1,378.....				15,632 c. c.
Typhoid Vaccine outdated and thrown out.....				1,002 c. c.
Typhoid Vaccine Manufactured (new).....				6,358
Typhoid Vaccine Distributed (new).....				936
Typhoid Vaccine on hand July 1, 1923.....				5,422 c. c.
COSTS:				
Personal Service.....				\$5,211 58
Laboratory Supplies.....				1,505 96
Laboratory Equipment.....				339 54
Other Expenses and Travel.....				619 68
TOTAL.....				\$7,676 76

Since moving into the new laboratory in December, 1921, the schedules of routine administrative procedure and rules and regulations for conduct have been very easy of enforcement. The enthusiastic response to an almost military discipline has made it possible to increase the number of examinations per scientific person very materially with no apparent effort on the part of the personnel. By referring to Chart III, showing number of specimens per person per month, it will be noted that in the fall of 1920, and again in the fall of 1921, the number of specimens per person per month ran up to about 1,000. Constant effort was needed in order to keep pace with the material that was coming in.

At the Houghton laboratory there was no great increase in work in 1919. The laboratory was located in the chemistry building of the Michigan School of Mines. In the spring of 1920, the laboratory was destroyed when the chemistry building burned, and the laboratory service in the northern peninsula was temporarily discontinued. It was reopened again for water, gonorrheal and tuberculosis examinations in May in the Quincy Mine

Dispensary at Hancock, and continued in operation there until the fall of 1921, when the new chemistry building in the School of Mines was completed. There was a steady increase, as indicated by Chart IV of the Houghton laboratory, with a maximum in the month of December, 1923. Necessarily, the use of the laboratory is much less general in the pioneer communities of the northern peninsula than in the more urban centers of southern Michigan, but there is every reason to believe that there will be a steady increase of work of the northern peninsula laboratory.

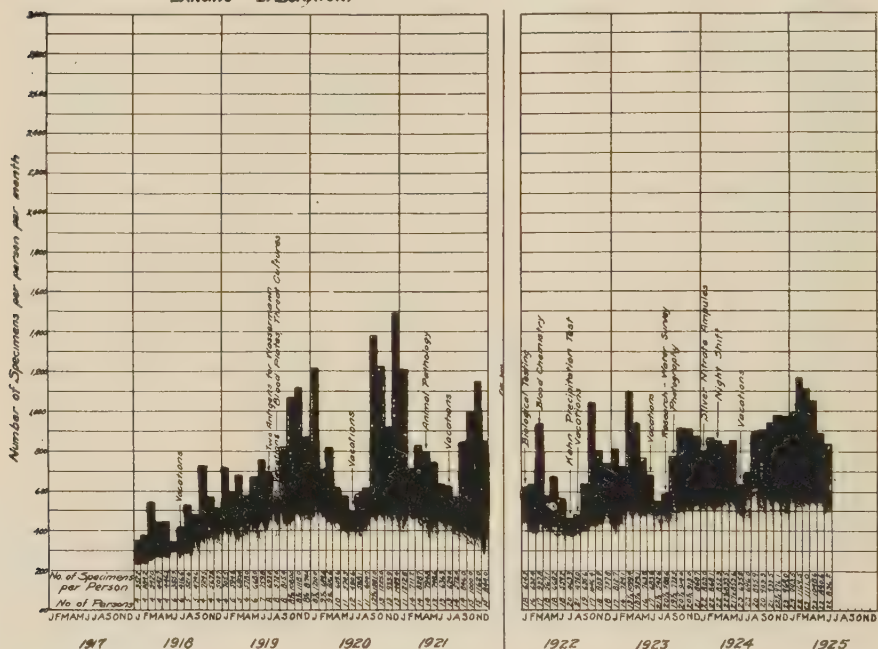


Chart III

During the period of development of the state laboratories from 1919 to 1923, there have grown up several municipal laboratories, notably, in Pontiac, Bay City, Saginaw, Battle Creek, Kalamazoo, Jackson, Grand Rapids, Port Huron and Flint, which in the aggregate do very nearly as much work as the Lansing laboratory on routine infectious disease diagnoses. This is as it should be. The central state laboratory at Lansing should gradually become a referee and research organization which sets the standards for maintenance of accuracy and dependability for the municipal laboratories. However, there will always be certain rural districts which will have to depend upon the state central or branch laboratories for service until the organic laws of the state are changed so that there will be district or county health units which can afford to maintain small diagnostic laboratories to care for the local work necessary in the control of quarantine.

The reasons for this unprecedented development might be summed up in the word "education." An analysis of various factors which influenced the growth in order of their magnitude, are in the mind of the director of laboratories, arranged as follows:

The practicing physician who was discharged from the army wished to put into use the many conveniences that he had had in the base hospitals. He turned to the state laboratory for this service and when he was met with a spirit of cooperation and secured accurate and dependable results, made

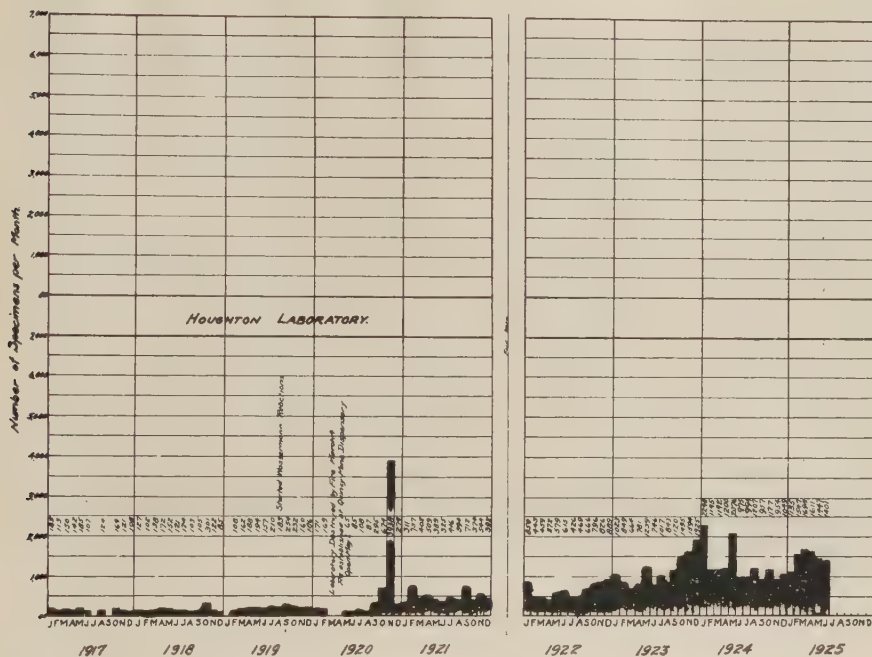


Chart IV

laboratory determinations a part of his routine examination. The returned army officer's reports to colleagues who had not been in service, stimulated them to send in material to aid in making diagnoses.

The next most potent factor was the abolition of the fee system. Physicians who did not feel justified in putting their patients to the expense of laboratory tests now began to use the laboratory routinely.

While these two factors in themselves would have made for a gradual increase in the work of the laboratory, the development was accelerated by the field work of members of the department of health. The department had several agencies which came in contact with the medical profession and the public. These agencies all urged laboratory information in the diagnosis of diseases and continually told the medical profession and the public that this work could be secured from the state laboratory. The traveling clinic

reached many medical societies and hundreds of individual doctors. The Bureau of Education by a series of circular letters, by personal representation and by lectures to the public disseminated information as to the use of the laboratory. Members of the laboratory staff went into the field in the fall of 1920 and cultured throats of thousands of children to demonstrate the presence of diphtheria carriers. At that time Michigan occupied the unique position of having the highest death rate from diphtheria of any state in the Union or any country in the world. As a result of these carrier surveys, health departments and health officers have followed the diphtheria cases and contacts very much more closely, which has increased the specimens for diagnosis of throat infections and will continue to do so.

As interesting commentary upon the extent to which the laboratory is used by the physicians and which in turn is an index of the amount of service done for the public, it is pointed out that in 1918, 1,700 physicians were recorded in the files as having had some work done. On December 31, 1923, a record for the year showed that 4,750 doctors had used the laboratory. As there are only about 6,000 registered physicians in Michigan, the director feels that practically every individual that would use a laboratory is getting some service from the State Department of Health Bureau of Laboratories.

Contemporary State Laboratories

While the development of the Lansing laboratory is unprecedented and exceeds the greatest relative development of any other state laboratory, all other laboratories have experienced a similar increase in volume of work done. New York still leads, with Michigan second, and Massachusetts third. The sixteen diagrams (Chart V) graphically show the increase in work in the laboratories of the states from which we had replies to the questionnaire sent out on this subject.

The consensus of opinion of the laboratory directors is that the increase in the demand for laboratory service is based upon education in medical matters that the public received during the war and the education of the medical profession in the use of diagnostic laboratories in the base hospitals of the army.

At the meeting of the 38th annual conference of the State and Provincial Health Officers held at Lansing in May, 1923, Doctor B. U. Richards, Commissioner of Health, Rhode Island, made a very comprehensive report on service of the state public health laboratories. Doctor Richards was acting chairman of a committee appointed by the president of the association. Part of his report was printed in the proceedings of the conference. The tabulated comparison of the work of the various laboratories from which he received replies is here introduced (Table III) to illustrate the character of the laboratory work that is now being done in the United States in the control of communicable diseases and service that is being given to the medical profession.

Doctor Vincent of the Rockefeller Foundation has been quoted as saying, "The extent to which a public diagnostic laboratory is utilized is one index of the intelligence, alertness and socialmindedness of the profession in the area which the laboratory serves."

The five years just passed have seen a public health awakening, and the next decade will unquestionably show a marked increase in the number of years of life expectancy. The public health laboratory will have no small part in extending the life of man.

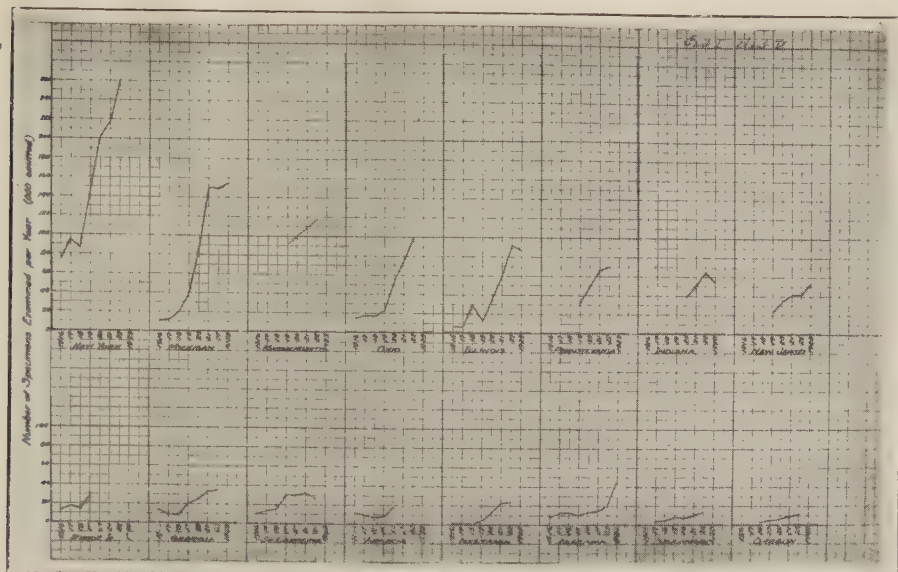


Chart V

CHAPTER III

THE ORGANIZATION OF THE BUREAU OF LABORATORIES

By far the most difficult task of perfecting a working organization and at the same time keep pace with the rapid development, was the selection of the personnel. With one or two exceptions no college or university in the United States trains students for the work of a public health laboratory. Courses are offered in fundamentals of chemistry, bacteriology and serology, but little attention is paid to the technology of these sciences in relation to diagnostic laboratory procedure. It has been the custom of laboratory directors to take graduates and train them over a period of two or three years.

The first step was to get an adequate salary for a skeleton organization which would be used for a training cadre. In 1919 the only really qualified person employed in the Michigan Department of Health laboratories was the chemist who did the medico-legal work. The new bacteriologist was selected

from available material on account of hospital experience and work in the base hospital laboratory during the war. The immunologist was selected because of his training and experience in the laboratory of the City of New York and the fact that he wished to do research in immunology using routine material. With these three individuals as a nucleus, university graduates were taken on and trained to do the technical work of the laboratories.

The next step was the reorganization of the records. They had been kept alphabetically in cross files between doctor and patient. To simplify the current records and to correlate the material from day to day so that at any time the distribution of the work could be determined, a geographical file was installed which was divided into counties, cities and villages, and the patient's file was discontinued. The patient's record was kept under the doctor's name and was filed in the city. If therefore, the city and the patient's name without the doctor's name were given, the information could be located. If the doctor's name and patient's name were given, the information could be found. The only occasion which could arise where the result could not be located would be where only the patient's name was available. This was so infrequent that it was of no consequence. It has occurred two or three times a year.

With the records in good shape the development of the service divisions was of course of paramount importance. Media production is the foundation of any diagnostic laboratory, and this was given special attention by the assistant bacteriologist. Animal care and glassware preparation bearing about equal relation to the laboratories as a whole, were developed satisfactorily only after moving into the new laboratory. The two other service divisions, the technical photography and instrument work, are rather recent additions, but they have already proved to be factors in facilitating the work of the technical divisions.

As will be noted in Chart VI, the Division of Microbiology is the pyramid about which the other divisions are built. It is the background of infectious disease control. Diagnostic bacteriology handles the bacteriology of transudates and exudates, typhoid diagnosis, diagnosis of throat infections, tuberculosis and gonorrheal examinations. The sanitary bacteriology does the milk and water work for the Bureau of Sanitary Engineering. Biological testing is done and reported for the Division of Antitoxin Distribution through the Division of Administration. All biologicals distributed by the Michigan Department of Health are tested for safety and potency before being distributed. To utilize any spare time that may occur on account of variable incoming work, the biological testing section makes typhoid vaccine for free distribution. The research section will be covered in another chapter as it bears a rather intricate relationship to the Division of Microbiology. The Division of Clinical Pathology grew up in the Division of Microbiology, but with the increased number of specimens for physiological chemistry, which were received from the institutions following the ruling of the State Ad-

ministrative Board, requiring the State Department of Health to supervise the medical service in penal and correctional institutions, the division was set off as a unit. The work in blood chemistry was also a very decided stimulus to the development of this division. The Division of Immunology was developed primarily to take care of the serum diagnosis of syphilis. It is sufficient to say that the work of this division is not only extremely accurate and dependable, but the members of the staff have done some very creditable research work in immunology, details of which will be found in the chapter on Research and Special Investigation.

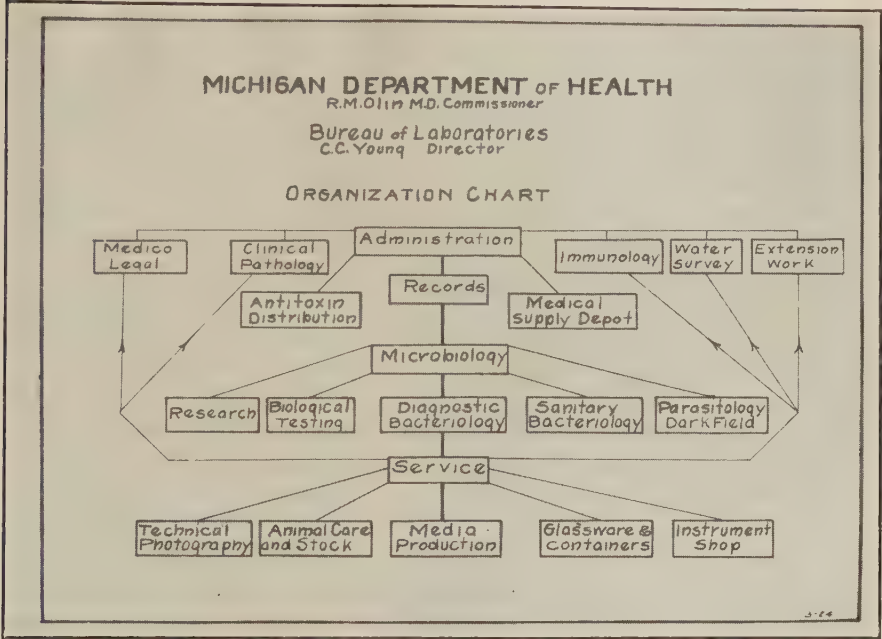


Chart VI

Of the auxiliary divisions, the Medico-legal Division makes analyses for county attorneys in the prosecution of murder cases and the investigations of the Michigan Department of Public Safety. The Water Survey Division was started in July 1923 to make a thorough survey of the mineral water in Michigan. The economic side of the question in relation to the work of the Bureau of Sanitary Engineering is a matter of first consideration. The second is the iodine content of the waters of Michigan in relation to the prevalence of simple goiter, which is being studied intently at the present time. The Extension Division, the Antitoxin Distribution Division and the Medical Supply Depot are covered in the appendix.

A study of the organization chart will show that the administrative responsibility passes from the director of laboratories to the chief of the Division

of Microbiology, who is the assistant to the director, and is essentially responsible for the records, and that the service divisions operate through the same administrative channel. Service reaches back to the other divisions without any administrative responsibility on the part of the chiefs of the divisions.

The first of each month the duty roster (see page 23) is posted on the bulletin board in each major division of the laboratory. This is subject to change without notice. It is felt that this is an advantage, not only to the director but to the personnel, to change individuals from one job to another, thus avoiding the habits of mind that lead to establishing ruts both in mental attitude and technical performance. To facilitate these changes, desks are permanently equipped and reserved for each type of work; one for typhoid diagnosis, one for throat infections, another for tuberculosis examinations, and so forth. Given the proper direction sheets, laboratorians experience no difficulty in orienting after a change. The duty roster for the month of January is given as an illustration. The educational value of this changing of work cannot be over estimated and the safety factor for overloads is increased to a maximum, for practically every individual in the course of a few months will be able to help out in any type of work.

To accomplish results by this method one must have carefully selected the personnel. The junior bacteriologist or serologist must have fundamental training in bacteriology, biology and chemistry and should have histology and physiology. Therefore, a Bachelor's degree in science is required for applicants before they are considered for a position. The new worker in the laboratory is put on three months' probation to see if he develops the proper attitude of mind toward the service of a diagnostic laboratory. This attitude cannot be exactly defined. To be a successful laboratorian, the worker must constantly focus on the sick person at the other end of the line, rather than upon the purely technical problem of getting a result. Accuracy and dependability are of prime importance and cannot be sacrificed for speed, but to be of any value, diagnostic laboratory results must reach the doctor in time to be of service to the patient.

The budget of the Bureau of Laboratories for 1923 (see page 24) was made out by the commissioner after conferring with the director of laboratories and the schedule of salaries fixed for the year. Vacancies in the more responsible positions in the laboratory are filled from the understudies in each division. This is the only possibility of a raise in salary. If vacancies occur among the junior bacteriologists they are filled from the outside from university graduates. To facilitate bookkeeping, each position is given a number which appears at the left of the column in the budget. On account of numerous changes during the year, titles of individuals in the duty roster sometimes bear little relation to the title appearing in the budget.

Duty Roster

January 1, 1924

ADMINISTRATION	C. C. Young, Director.
MICROBIOLOGY	M. Crooks, Bacteriologist.
1. Diagnostic Bacteriology:	
Diphtheria diagnosis	P. C. Yull, Assistant Bacteriologist. J. Stevens, Junior Bacteriologist. L. Gerstner, Senior Bacteriologist.
Typhoid diagnosis	P. C. Yull, Assistant Bacteriologist. L. D. Henry, Senior Bacteriologist. L. Gerstner, Senior Bacteriologist.
General bacteriology	H. Baker, Junior Bacteriologist. M. Rouse, Senior Bacteriologist.
Tuberculosis diagnosis	M. Rouse, Senior Bacteriologist. E. Shultis, Junior Bacteriologist.
Gonorrheal examinations	H. Baker, Junior Bacteriologist. M. Rouse, Senior Bacteriologist. A. Worcester or N. Baker, Technicians.
2. Sanitary Bacteriology	A. Exworthy, Senior Bacteriologist. H. Baker (part time), Junior Bacteriologist.
3. Research	M. S. Marshall, Research Bacteriologist.
4. Biological Testing and Vaccine Production	P. C. Yull, Assistant Bacteriologist.
5. Parasitology	S. R. Johnson (part time), Dept. of Agri- culture.
Darkfield examinations	P. C. Yull, Assistant Bacteriologist. L. Gerstner, Senior Bacteriologist.
Stock Cultures	E. Shultis, Junior Bacteriologist.
IMMUNOLOGY	R. L. Kahn, Immunologist.
Research	R. L. Kahn, Immunologist. P. L. Kendricks, Assistant Immunologist.
Kahn Precipitation test	J. Landau, Senior Serologist. E. White, Technician. B. Bartholomew, Technician.
Wassermann test	D. Jenks, Junior Serologist. M. Lowe, Technician. B. Bartholomew, Technician. R. Wood, Laboratory Worker.
WATER SURVEY	E. F. Eldridge, Analytical Chemist.
MEDICO-LEGAL	C. L. Bliss, Analytical Chemist.
CLINICAL PATHOLOGY	A. B. Haw, Physiological Chemist.
Chemical diagnosis	A. B. Haw, Physiological Chemist. A. Exworthy (part time), Assistant Chemist.
Hematology	E. Shultis, Junior Bacteriologist.
RECORDS	C. Thoman, Chief Clerk.
Correspondence	F. Cleland, Stenographer.
Information	M. Elliott, Clerk.
Immunological Reports	G. Atkinson, Stenographer.
Bacteriological Reports	J. Finch, Clerk.
Chemical Reports	C. Thoman, Chief Clerk.
Wassermann Card File	E. Marvin, Clerk.
Sputum Card File	M. Elliott, Clerk.
Gonorrhea and Typhoid Card File	A. Milenz, Clerk.
Throat Infection Card File	T. Trerice, Clerk.
Mail Entry	A. Milenz, Clerk.
SERVICE	
1. Media Production	L. D. Henry, Senior Bacteriologist.
Standardization	M. S. Marshall, Research Bacteriologist. N. Baker, Technician. A. Worcester, Technician.
2. Glassware and Containers	I. Teachout, Forewoman. D. Magee, Laboratory Worker.
Glassware	R. Brier, Laboratory Worker. C. Frasier, Laboratory Worker.

3. Instrument Shop R. L. Burd, Expert Mechanic.
4. Technical Photography L. C. Hulbert, Photographer.
5. Animal Care and Stock O. Burcham, Caretaker.

EXTENSION WORK (See Division of Instruction, Appendix IV)

ANTITOXIN DISTRIBUTION

- Records B. Macgregor, Stenographer and Bookkeeper.
 Shipping and Receiving C. Selden, Clerk.
 T. Trerice, Typist.

MEDICAL SUPPLY DEPOT

- G. Klawitter, Storekeeper.
 M. Kneebone, Assistant Storekeeper.
 E. Pitt, Bookkeeper.
 B. Siebert, Bookkeeper.
 A. Bruce, Laborer.

BUDGET

July 1, 1923 to July 1, 1924

Personal Service	\$66,310.00
Travel	1,000.00
Other Contractual Service	1,500.00
Supplies	11,000.00
Equipment	9,925.00
	\$89,735.00

Budget

PERSONNEL—LABORATORY

No.	Title	Present Salary	Proposed Salary
1.	Director of Laboratories	\$4,000.00	\$4,500.00
2.	Immunologist	3,500.00	3,500.00
3.	Assistant Immunologist	1,800.00	1,800.00
4.	Assistant Immunologist	1,800.00	1,800.00
5.	Assistant Immunologist	1,650.00	1,650.00
6.	Assistant Immunologist	1,200.00	1,350.00
7.	Technical Assistant	1,500.00	1,500.00
8.	Bacteriologist	2,950.00	3,000.00
9.	Assistant Bacteriologist	2,250.00	2,400.00
10.	Assistant Bacteriologist	1,650.00	1,800.00
11.	Assistant Bacteriologist	1,400.00	1,400.00
12.	Assistant Bacteriologist	1,200.00	1,200.00
13.	Assistant Bacteriologist	1,200.00	1,350.00
14.	Assistant Bacteriologist		1,200.00
15.	Assistant Bacteriologist	1,500.00	1,500.00
16.	Stenographer	1,800.00	1,800.00
17.	Stenographer	1,300.00	1,300.00
18.	Stenographer	1,200.00	1,200.00
19.	Clerk	1,200.00	1,500.00
20.	Clerk	1,000.00	1,100.00
21.	Clerk	1,100.00	1,100.00
22.	Clerk	1,000.00	1,000.00
28.	Assistant Bacteriologist	2,500.00	2,500.00
29.	Assistant Bacteriologist	1,200.00	1,200.00
30.	Preparator	960.00	960.00
31.	Preparator	960.00	960.00
38.	Preparator	960.00	1,200.00
32.	State Chemist	2,750.00	2,750.00

Budget.—Concluded

No.	Title	Present Salary	Proposed Salary
33.....	Assistant Chemist.....		\$2,500.00
34.....	Parasitologist.....	\$2,600.00	2,600.00
35.....	Animal Caretaker.....	1,500.00	1,500.00
36.....	Janitor.....	1,200.00	1,200.00
37.....	Janitor.....	1,000.00	1,000.00
39.....	Dishwasher.....	780.00	780.00
40.....	Dishwasher.....	780.00	780.00
41.....	Dishwasher.....	780.00	780.00
42.....	Bacteriologist (Houghton Laboratory).....	2,850.00	2,850.00
43.....	Technician (Houghton Laboratory).....	1,400.00	1,400.00
44.....	Janitor (Houghton Laboratory).....	300.00	300.00
45.....	Dishwasher (Houghton Laboratory).....	300.00	300.00
46.....	Assistant Bacteriologist (Houghton Lab.)...	1,500.00	1,800.00
			\$66,310.00

ANTITOXIN OPERATION—APPROPRIATION, \$60,000.00

No.	Title	Present Salary	Proposed Salary
23.....	Chief Clerk.....	\$1,800.00	1,800.00
24.....	Technician.....	2,000.00	2,250.00
25.....	Research Assistant.....		2,500.00
26.....	Shipping Clerk.....	1,500.00	1,800.00
27.....	Clerk.....	1,200.00	1,200.00
			\$9,550.00

CHAPTER IV

RESEARCH AND SPECIAL INVESTIGATIONS

With the extension of the field of the public health laboratory into the realm of bacteriology of transudates and exudates as an aid to physicians in the diagnosis of infections, we are often precipitated into an indefinite field of theoretical considerations. Is the organism found pathogenic? Time and time again we are unable to follow out leads which might bring us to a solution of some problem of etiological significance of some organism. As an illustration, if we find a gram negative encapsulated rod in urine from a catheterized kidney, we are unable to follow up the case and correlate it with other similar cases. The press of the daily routine absolutely precludes work on these problems. Consequently it was deemed advisable to add to the staff a research bacteriologist whose duties would be first, to check up new methods of examination appearing in the literature which might be

applicable to the routine procedure of a diagnostic laboratory and which might improve the service; second, to standardize the media to meet the growth requirements of organisms; third, to take over and study any special problems that arose which were significant enough to warrant special investigation. In the Division of Immunology, sufficient leeway of personnel has been maintained so that the chief of the division and his first assistant can devote their time largely to controls of the Wassermann test including its dependability and do research on the Kahn precipitation test and other serological methods for the diagnosis of syphilis.

Every individual in the laboratory is encouraged to do some research or make some special investigation which will act as a background for the daily routine or furnish scientific information for use of other bureaus. It is believed that every piece of work of this character which develops initiative and requires a certain amount of original thinking, adds to the usefulness of the worker to the organization and improves the character and quality of the results obtained. It is a splendid means of letting recent graduates from universities and colleges find out what they do not know.

After the reorganization in 1919, the following special investigations and special research problems were completed by members of the staff of the Michigan Department of Health laboratory. The two outstanding problems solved are the use of the blood plate in the routine diagnosis of throat infections other than diphtheria, by M. Crooks, and the application of precipitation tests to the diagnosis of syphilis, by R. L. Kahn.

1. Leprosy, Case Report: Abram Getz, Benton Harbor, Mich.—C. C. Young, 1919.
2. Notes on the Renovation of Feathers.—C. C. Young, 1919.
3. An Outbreak of Septic Sore Throat, Freeport, Mich.—C. C. Young and A. G. Boyd, 1919.
4. Comparative Studies of Diphtheria Cultures on Loeffler's Medium with the Original Swabs Transported by Mail.—C. C. Young and M. Crooks. Read, Am. Pub. Health Assn., San Francisco, Calif., 1920. Am. J. Pub. Health, Vol. XI. (Mar.) 1921.
5. Incidence of Beta Hemolytic Streptococci in a Scarlet Fever Outbreak at Nazareth Academy, Kalamazoo, Mich.—C. C. Young and M. Crooks, 1920.
6. Study of the Bacterial Flora of New-born Infants.—C. C. Young and M. Crooks, 1920.
7. A Study of Active Immunization Against Diphtheria in the Michigan Home and Training School, Lapeer, Mich.—C. C. Young, 1920. Noted A. M. A.
8. Complement vs. the Amboceptor Titrations in Wassermann Test.—R. L. Kahn. J. of Lab. and Clin. Med. 6:153. 1920.
9. Comparison of Bacterial Count and the Spore Test.—Alice Exworthy, 1920.
10. A Simple Method for the Removal of Natural Amboceptor from Human Sera.—R. L. Kahn. J. of Lab. and Clin. Med., VI, 218, 1921.
11. The Wassermann Test in the Public Health Laboratory.—R. L. Kahn. Am. J. Pub. Health, XI, 410, 1921.
12. Optimum Conditions of Fixation of Complement in Wassermann Test.—R. L. Kahn. Arch. of Derm. and Syphil. IV, 358, 1921.
13. The Wassermann Test and Its Interpretation.—R. L. Kahn. J. of Lab. and Clin. Med., VI, 579, 1921.
14. The Quantitative Relation between Complement and Complement Fixing Antibody.—R. L. Kahn. Proc. Soc. for Exp. Biol. and Med., XVIII, 170, 1921.
15. On the Persistence of Complement Fixing Antibodies in the Serum of Rabbits Immunized with Purified Proteins.—R. L. Kahn. Proc. Soc. for Exp. Biol. and Med., XVIII, 294, 1921.

16. Studies on Complement Fixation I: The Rate of Fixation of Complement at Different Temperatures.—R. L. Kahn. *J. of Exp. Med.*, XXXIV, 217, 1921.
17. Studies on Complement Fixation II: Velocity of Fixation of Complement in the Wassermann Test.—R. L. Kahn and R. M. Olin, Jr. *J. of Inf. Dis.*, 29:630, 1921.
18. Studies on Complement Fixation III: The Effect of Heat on Complement Fixing Antibodies.—R. L. Kahn, S. R. Johnson and A. G. Boyd. *J. of Inf. Dis.*, 29:651, 1921.
19. Studies on Complement Fixation IV: On the Affinity of Sheep—Corpuscles for Antisheep Hemolysin.—R. L. Kahn and D. S. Lyon. *J. of Inf. Dis.*, 29:651, 1921.
20. A Bacteriological Study of a Typhoid Outbreak.—C. C. Young and M. Crooks. Read Lansing Branch Society American Bacteriologists. *Abst. Bact.*, 5:208, 1921.
21. Public Health Problem of Throat Infections.—C. C. Young. Read Public Health Section, Mich. State Med. Soc., Bay City, Mich., 1921.
22. Frost's Little Plate Method Applied to Routine Diphtheria Diagnosis.—Frances B. Hawkins. Read Public Health Section, Mich. State Med. Soc., Bay City, Mich., 1921.
23. Classification of Organisms of the Colon Group Isolated from Michigan Wells.—Alice Exworthy. 1921.
24. On Routine Reporting of Types of Streptococci.—C. C. Young and M. Crooks. Read Society of American Bacteriologists, Philadelphia. *Abst. Bact.*, 6:32, 1922.
25. Williamston Typhoid Epidemic.—C. C. Young, Albert Jewell, San. Eng., P. C. Yull, Bacteriologist. 1921.
26. Experiments to Determine the Value of the Tuberculin Test as a Means of Early Diagnosis in Guinea Pigs.—P. L. Kendrick. 1921.
27. A Comparison of the Hydrogen-ion Determination Method of Coolidge With the Bacterial Count of Milk.—Alice Exworthy. 1921.
28. A Study of Klebs-Loeffler Bacilli Grown on "Difco" Dehydrated Serum Plus Tellurite 1%.—M. Crooks. 1922.
29. Studies on Complement Fixation V: The Titre vs. Fixability of Complement.—R. L. Kahn and Ethel D. White. *J. of Inf. Dis.*, 30:313. 1922.
30. A Simple Quantitative Precipitation Reaction for Syphilis—Preliminary Communication.—R. L. Kahn. *Arch. Derm. and Syph.*, 5:570. 1922.
31. A Simple Quantitative Precipitation Reaction for Syphilis—Second Communication.—R. L. Kahn. *Arch. Derm. and Syph.*, 5:734. 1922.
32. Effect of Dilution on Precipitation Reaction for Syphilis Proposed by Author.—R. L. Kahn. *Proc. Soc. Exp. Biol. and Med.*, 19:131. 1922.
33. Relation Between Serum and Antigen in Precipitation Reaction for Syphilis Proposed by Author.—R. L. Kahn. *Proc. Soc. for Exp. Biol. and Med.*, 19:295. 1922.
34. A Simple Quantitative Precipitation Reaction for Syphilis—Third Communication.—R. L. Kahn. *Arch. Derm. and Syph.* 6:332. 1922.
35. Requirements for Correct Wassermann Tests.—R. L. Kahn. *Abst. Bact.*, 6:157. 1922.
36. Quantitative Relation Between Antigen and Antibody in Complement Fixation.—R. L. Kahn and S. R. Johnson. *Abst. Bact.*, 6:157. 1922.
37. Studies on Complement Fixation VI: The Velocity of Fixation of Complement with Bacterial Antigens.—R. L. Kahn and S. R. Johnson. *J. of Inf. Dis.*, 31:416. 1922.
38. Studies on Complement Fixation VII: The Determination of the Optimum Amount of Antigen in Complement Fixation Tests.—R. L. Kahn and S. R. Johnson. *J. of Inf. Dis.*, 31:416. 1922.
39. Studies on Complement Fixation VIII: The Effect of Inactivation on Complement-Fixing Substances in Syphilitic Serum.—R. L. Kahn and S. R. Johnson. *J. of Inf. Dis.*, 31:416. 1922.
40. A Simple Quantitative Precipitation Reaction for Syphilis.—R. L. Kahn. Read Mich. Academy of Science, 1922.
41. Viability of *B. Coli*.—C. C. Young. Read Mich. Academy of Science, 1922.
42. Cultural and Agglutination Studies with Special Reference to the Widal Reaction.—P. L. Kendrick and M. Crooks. Read Mich. Academy of Science, 1922.
43. Report on Experimental Work with Complement Fixation, Employing Sera from Tubercular Subjects.—S. R. Johnson, 1922.

44. Laboratory Service in Public Health Work.—C. C. Young. Read, Public Health Section, Mich. State Med. Society, 1922.
45. A Comparison of the Use of Glycerinated and Non-glycerinated Swabs in Diphtheria Diagnosis.—Helen Gunn, 1922.
46. The Public Health Value of the Kahn Test for Syphilis. A Preliminary Report Based on 5000 Examinations.—C. C. Young. J. A. M. A., 79:1670. 1922.
47. Syringe for Schick Testing.—C. C. Young and M. Crooks. J. A. M. A., 78:651. (Mar. 4) 1922. Fig. 2.
48. Modified Keidel Tube for Collecting Blood Specimens.—C. C. Young. J. A. M. A., 79:739. (Aug. 26) 1922. Fig. 6.
49. The Kahn Test for Syphilis in a Public Health Laboratory.—C. C. Young. Am. J. Pub. Health, 13:96. 1923.
50. Castor Pomice Poisoning.—S. R. Johnson. J. Am. Vet. Med. Assn., Vol. 58. (May) 1923.
51. Observations on the Viability of the Bacterium Coli Group under Natural and Artificial Conditions.—C. C. Young and M. Greenfield. Am. J. Pub. Health, 13:270. 1923.
52. Observations on Kahn Precipitation Reaction for Syphilis.—R. L. Kahn. Am. J. of Syph., 7:389. 1923.
53. Kahn Precipitation Test for Syphilis—Improved Procedure.—R. L. Kahn. Proc. Soc. for Exp. Biol. and Med., 20:325. 1923.
54. Dilution of Antigen for Wassermann Test.—R. L. Kahn. Proc. Soc. for Exp. Biol. and Med., 20:332. 1923.
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56. Employment of Different Antigens in the Kahn Precipitation Test.—R. L. Kahn and W. W. Duemling. Proc. Soc. for Exp. Biol. and Med., 20:492. 1923.
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58. Quantitative Relation between Complement Fixation and Agglutination.—Pearl L. Kendrick and R. L. Kahn. Abst. Bact., 7:25. 1923.
59. Application of Author's Precipitation Test to Spinal Fluids.—R. L. Kahn. Proc. Soc. for Exp. Biol. and Med., 21:76. 1923.
60. Effect of Contamination and Age of Serum on Kahn Precipitation Test.—P. L. Kendrick and R. L. Kahn. (In Press.)
61. Some Recent Observations on Author's Precipitation Test.—R. L. Kahn. (In Press.)
62. Role of Concentration in the Serology of Syphilis by Precipitation.—R. L. Kahn. (In Preparation.)
63. Quantitative Relation between Serum and Antigen in the Serology of Syphilis by Precipitation.—R. L. Kahn and J. L. Landau. (In Preparation.)
64. On Precipitation Test for Syphilis with Special Reference to the Kahn Reaction.—R. L. Kahn. (In Preparation.)
65. The Kahn Precipitation Test with Different Antigens.—P. L. Kendrick and R. L. Kahn. (In Preparation.)
66. Bacteriological Study of an Epidemic of Thirty Cases of Post tonsillectomy Infection.—P. L. Kendrick, Lansing Branch, Society of American Bacteriologists, 1923.
67. A Study of Beta Hemolytic Streptococci Recovered from Scarlet Fever Cases at Michigan Agricultural College, at Scarlet Fever Pavilion, Grand Rapids.—(In Progress.)
68. Bacteriological Examination of Excised Tonsils from State Industrial School, Lansing; State Public School, Coldwater; Michigan Reformatory, Ionia.—(In Progress.)
69. Investigation of the Intestinal Flora Including Bacterial and Intestinal Parasites of the Feces from Industrial School, Lansing.—(In Progress.)
70. Special clinical studies on the Kahn Precipitation Test in cooperation with the Department of Dermatology and Syphilis of the University Medical School, the aim being to determine (1) the exact status of the test in different stages of syphilis; (2) the immediate effect of individual treatments on the tests.—(In Progress.)
71. Experiments to Determine the Efficiency of the Electropure Milk Pasteurizer in Killing Pathogenic Organisms.—C. C. Young.

72. The Kahn Precipitation Test for Syphilis. (a) Two Years' Experience in a Public Health Laboratory.—C. C. Young. (b) Demonstrations.—R. L. Kahn. Am. Pub. Health Assn., Boston, 1923. (In Press.)
73. The Value of the Direct Smear in Diphtheria Diagnosis in a Public Health Laboratory.—M. Crooks and C. C. Young. (In Preparation.)
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79. Study of Alkanite as a Stimulant to Growth of Cocci, with the Idea of Testing its Applicability for Use in Culture Media.—P. C. Yull, 1923.
80. Study of Lactose Broth Plus Gentian Violet as a Means of Eliminating Spore Formers in Water Analysis.—Alice Exworthy.
81. Investigation of Diarrhea Outbreak at Michigan Home and Training School, Lapeer, Mich., 1923.
82. The Determination of Iodin in Water by Charcoal Absorption.—E. F. Eldridge, 1923.
83. The Determination of the Inhibiting Effect of Potassium Oxalate and Potassium Sulphate on Organisms commonly Recovered from Blood Cultures.—L. D. Henry, 1923.
84. The Instability of Dextrin in Culture Media.—M. S. Marshall and L. D. Henry, 1923. (In Preparation.)
85. A Note on Medalia's Method of Colorimetric pH Determination.—M. S. Marshall, 1923.
86. A Report on Immersion Oils.—M. S. Marshall, 1923.
87. Testing of Dyes in Cooperation with the Committee on Standardization of Dyes, Society of American Bacteriologists.—M. S. Marshall, 1923.

CHAPTER V

PLANS FOR THE FUTURE

Before predicting what is going to happen in the development of state public health laboratories we must consider the trend of development of state health departments. As these departments become more and more socialized and greater demands are placed upon them to function as an aid in keeping the people well, public health laboratory work must lead in scientific research and must constantly follow the development by doing the routine tasks which constitute the control measures.

It is freely predicted in the light of the development of the Lansing laboratory that it will be necessary to decentralize these routine tasks. The geography of Michigan and its railways indicate that the most advantageous locations for branch laboratories in order of their importance are Grand Rapids first, Bay City or Saginaw second, and Wayne County third.

Specific prevention of diseases by producing artificial immunity is in its infancy. We have a few known specifics which are at the present time distributed by state health departments. It is the intention to extend the list of products distributed by the Michigan Department of Health by adding silver nitrate for the prevention of gonorrheal ophthalmia. It is within the reasonable expectancy of scientific workers that measles and scarlet fever will be brought into the same realm as diphtheria and smallpox.

Unquestionably the laboratories of the future will concern themselves with the development of immunology. It is to be expected that control of the production and distribution of non-specifics or alleged specific serums, bacterins and viruses will be brought under the supervision of state departments of health.

Every section of the country will have its own problems. In the south, hookworm and malaria will be concentrated upon; in Michigan, throat infections, goiter, seasonal asthma and intestinal infections other than typhoid.

With these added interests there is no doubt in the mind of the writer that the next decade will see even greater development in the public health laboratory service to the medical profession and through them to the public.

TABLE III

[illegible]

PART II

OUTLINE OF METHODS OF ROUTINE PROCEDURE

(Revised January 1, 1925)

RULES AND REGULATIONS

"Any employee of the Michigan Department of Health divulging information pertaining to any reports or records of the department may consider such conduct sufficient grounds for dismissal."—Dr. R. M. Olin.

Requisitions for biologicals shall be referred to the Chief of the Division of Biological Distribution.

No person shall have access to the vault except as indicated by the Chief Clerk or director's stenographer.

Refer all inquiries pertaining to laboratory results to the person officially responsible for reporting the work about which inquiry is made.

The official hours on duty are from 8 a.m. to 12 noon, and from 1 p.m. to 5 p.m., except Saturday afternoon which is a holiday when work permits.

Employees will have 24 days off duty each year including time off for sickness. No employee is entitled to leave of absence (except for serious illness or other emergency) unless he has been in the service of the department six months and has filled out leave of absence slips and has had same approved by the Bureau head. Employees will have only two weeks in sequence for vacation.

Notify the Information Desk when unable to report for duty.

While on duty, all employees of the laboratory shall wear rubber heels, and a protective gown. Gowns are supplied by the Preparation Division, mezzanine floor, and may be exchanged on Monday and Thursday from 8 to 9 a.m.

Lockers are provided for wraps.

Written requisitions for supplies from store must be on Information Desk Tuesday and Friday for delivery Wednesday and Saturday.

Make all purchases through the Auditor and give delivery slips showing receipt of purchase.

Place broken glassware and infectious material in receptacles provided. *Do not put broken glassware or infectious material in waste paper baskets.*

Use the telephones for state business only. Memoranda of phone calls will be placed in the employee's mail box.

Visitors and observers are limited to persons interested in laboratory work. Sightseers and callers will be referred to the Information Desk.

The department library is on the 6th floor. A librarian is on duty from 8 a.m. to 5 p.m. Current scientific magazines are on a table in laboratory foyer. These periodicals are loaned to the laboratory for one week, but may be subsequently borrowed from the library. Books and magazines may be secured from M. S. C., U. of M., and John Crearar Library by sending written requests to the Bureau of Education.

Permanent immunity can be conferred against diphtheria, typhoid fever, and smallpox. The department assumes no responsibility if the individual neglects to protect himself against the infection.

Every member of the laboratory personnel is responsible for the information contained in the bulletin, all memoranda on the bulletin board in the lobby and all memoranda in the room in which he works.

CHAPTER VI.

DIVISION OF RECORDS

Candace Thoman, B. S., Chief Clerk

Methods of Filing

All records of the Bureau of Laboratories are filed geographically and are classified as follows:

I. Correspondence—Director's Secretary

1. Out-of-state, filed by state

2. State, filed by counties, except

Bureau of Laboratories (Lansing) by subject.

Inactive filed in transfer cases

Administration

Memoranda to Director

Memoranda to Laboratory Personnel

Memoranda to Medical Supply

Stores Requisitions

Monthly Reports

Annual Reports

Circular Letters

Postal Regulations

Guinea pigs and other animals

Current problems—those in which the Director is actively interested

Division Reports

Manuscripts

Consulting Pathologist

Research

Stock Cultures Received and Shipped

Biological Reports

Scientific Societies

Building Equipment, including inventories

Branch Laboratory (Houghton)

3. Personnel File

Application letters and recommendation letters, by name, including correspondence to and from employees.

Exceptions: 1. Budget and payroll memoranda in Director's desk.

2. Dr. Kahn's correspondence filed in desk in Wassermann Laboratory.

II. Records—Chief Clerk

1. Card file, recording examinations of

- (a) Throat and Nasal swabs for B. diphtheria and streptococcus; also ear swabs beginning February 1923.
- (b) Blood for Wassermann reaction and Kahn precipitation test; reports for Welfare Commission are not recorded but original reports are filed in the vault under the name of the institution submitting the specimen.
- (c) Pus smears for gonorrhea, including urine
- (d) Sputum for B. tuberculosis
- (e) Blood, urine and feces for B. typhosus

NOTE: This file is in the Record room; the original blanks from which it is made are stored in the vault and kept in chronological order.

- (f) Institutional reports before July 1, 1923, are filed under the name of the Institution in transfer cases in the vault. After this date they are filed with the routine reports.

2. File of original laboratory reports is in the vault and consists of reports on

- (a) Transudates and Exudates
- (b) Blood, not otherwise classified
- (c) Urine, not otherwise classified
- (d) Water
- (e) Animal pathology—transferred to Department of Agriculture, October 1, 1923
- (f) Miscellaneous specimens—includes toxicology, milk, feces for parasites and tuberculosis, specimens referred to other Departments
- (g) Blood for Wassermann and Kahn from Maternity Hospitals and Child Caring and Placing Agencies

Exceptions: 1. Supplementary water file in wooden file cases

- 2. Toxicology reports may also be found in correspondence filed under county or town.

3. Paid and collect telegrams filed separately and chronologically in the vault

4. Entry blanks

All except current entry blanks are filed chronologically in the vault.

5. Reports on unsatisfactory specimens are filed chronologically in transfer cases or at back of files.

- (a) Insufficient material
- (b) Broken or leaked out in transit
- (c) Insufficient information concerning name or address of physician submitting specimen.

Exception: Reports on blood for Wassermann which is hemolyzed or has leaked out are filed in the vault.

6. Slides for gonorrhea diagnosis are filed chronologically in cabinet in the record room.

III. Gonococcus Slide File

Pus smears examined for gonococci are filed in a cabinet provided for this purpose in the record room. All positive diagnostic slides are saved indefinitely. Negative slides are destroyed every six months. These slides are filed daily under the date appearing on the report of the physician. Slides are numbered in the laboratory in the upper right hand corner on side smear has been made. Blanks are given the same number. When slides are ready for filing, labels are placed on opposite side of smears, leaving laboratory number visible if possible.

1. Negative slides are labeled with laboratory number and *reporting* date.
2. Positive slides are labeled with reporting date, patient's name, doctor's name and address.
3. Extracellular smears are filed as positives.

NOTE: Only *one* positive smear is saved for filing. *All* negative smears are saved and filed. If two smears are received from same patient and one is positive and one negative, the positive slide only is filed.

Color Scheme

This laboratory has adopted a scheme of colors in order to differentiate the kinds of blanks used in the records and reports of the organization.*

Blue indicates swabs for diphtheria and streptococcus

Cherry indicates blood for Wassermann and Kahn precipitation test

Pink indicates blood examination other than Wassermann, Kahn and typhoid

Green indicates typhoid diagnosis

Cafe indicates urine examination (chemical)

Salmon indicates transudates and exudates (spinal fluid)

Gray indicates water, ice and milk examinations, and sewage examinations

Canary indicates sputum examinations

Brown indicates gonorrheal examinations

White indicates toxicological, unclassified and miscellaneous examinations

*A complete set of laboratory blanks may be found in Appendix F.

Mail Entry, Counting and Recording of Laboratory Reports

Entry blanks are used for recording the receipt of specimens, each entry giving the date and time of arrival, physician's name and address, number of specimens received and, when necessary, the kind of specimen. In case the physician submitting the specimen has not filled out an information blank furnished by the laboratory with his name and address, those of the patient, and a history of the case, such a blank is made out in the mailing room from any information which may accompany the specimen. All information sent by the doctor is saved and filed with the laboratory blanks. The time and date of receiving specimens is stamped on each blank. The specimens are to be entered and delivered to the laboratory promptly. Entries shall be recorded by the receiving clerks only. Great care and accuracy is to be exercised in making the entries as the entry blanks form a permanent record of all specimens received in the laboratory, together with the laboratory report on their examination. The reports will be classified and numbered as follows:

I. Throat swabs

Blanks accompanying throat swabs shall be stamped in the laboratory as follows: Diagnosis, Release, Carrier.

1. Diagnostic swabs are examined for B. diphtheria, streptococci and Organisms of Vincent.
2. Release swabs include release from diphtheria quarantine and release from quarantine for scarlet fever and isolation for septic sore throat.
3. Carrier swabs include routine admission and discharge throat swabs from hospitals; contacts with cases of diphtheria and septic sore throats; throat swabs from scarlet fever contacts; measles, influenza and pneumonia, unless there is a history of sore throat; and routine throat swabs from clinics.
4. B. diphtheria receives one number, streptococcus receives one number and Organisms of Vincent receives one number. If colored crayon laboratory number indicates two swabs, the report will be numbered accordingly. A virulence test receives one number and the result will be recorded on entry blank on which the swab was entered.

II. Sputum

1. B. tuberculosis receives one number.
2. Streptococci receives one number and is counted in the miscellaneous column.
3. Typing receives one number and is counted in the miscellaneous column.
4. The routine examination is for B. tuberculosis. A culture or animal inoculation may be made.

III. Feces

Feces will be numbered in accordance with information on the back of the laboratory blank. All examinations except for typhoid and dysentery are in the miscellaneous classification. Supplementary examinations will include procedure indicated on the back of the laboratory report.

IV. Blood

1. Blood count

This classification includes differential blood count, red and white counts and blood smears for malarial parasites. Each receives one number. Note blood smear for parasites when necessary.

2. Chemical blood

This classification includes sugar determination, nitrogen, etc., and each determination receives one number.

3. Widal reaction

This examination is for typhoid and receives one number.

4. Blood culture

The examinations will be numbered according to the information on the back of the laboratory blank. A positive blood culture is one showing any organism except contamination.

5. Supplementary examinations will include procedure indicated on back of report not appearing on final report.

V. Urine

1. Routine urine analysis receives two numbers, one for chemical and one for sediment examinations.

2. Each group of determinations in a qualitative urine report receives one number.

3. Bacteriological examinations will be numbered in accordance with the information on back of the report.

4. Supplementary examinations will include procedure other than that appearing on the final report.

VI. Water, Milk, Ice and Sewage

1. Water

(a) Each bacteriological analysis receives one number.

(b) Each chemical finding in chemical analyses receives a number.

2. Each bacteriological examination of milk receives one number.

3. Ice is classified as water.

4. Each sample of sewage receives one number. Extra tests receive additional numbers.

VII. Transudates and Exudates

This classification includes all body fluids not otherwise classified, that is, all swabs with pus, pleural fluids, spinal fluids, etc.

1. These reports will be numbered in accordance with information on the laboratory blank.
2. Supplementary examinations will include procedure on back of the final report.

VIII. Examination for Gonorrheal infections

1. Each smear examined for gonococci will receive one number.
2. Cultures for gonococci will be numbered as indicated on the blank.

IX. Wassermann Reaction and Kahn Test

1. A Wassermann test receives one number.
2. The Kahn Precipitation test receives one number.

X. Toxicological Examinations

This classification includes all specimens submitted to the chemist to be examined for poison. Examinations will be numbered as directed.

XI. Unclassified examinations

In this classification are included tissue for bacteriological examination and specimens not otherwise classified. Examinations will be numbered as directed. This classification should be kept as small as possible.

XII. Animal Pathology

This includes all veterinary examinations and was transferred to the Department of Agriculture, October 1, 1923.

XIII. Animal Inoculations

The animal inoculation card is sent through with autopsy report and is numbered and filed then in the Animal Inoculation Card File.

1. Each animal inoculation will be recorded on entry blank on which specimen was entered and will receive one number.
2. Each animal autopsy will receive one number and be counted as a supplementary examination. Exception: Virulence tests receive but one number.
3. Each vaccine report is recorded on entry blank in "Vaccine" column. Vaccine receives one number unless additional examinations are reported. These will be numbered and recorded as supplementary and the whole will be recorded on the entry blank on which the specimen was entered. If report says "Vaccine being prepared" do not number for vaccine until card is sent through.

Methods of Reporting Laboratory Examinations

I. General Considerations

1. Reports for Bureaus

Make two copies

- (a) for doctor submitting specimen
- (b) for files (to be filed under Bureau. Do not file under doctor's name)

All intra-departmental letters must be signed by the Director of Laboratories.

Bureaus:

Child Hygiene and Public Health Nursing

Records and Statistics

Institutional Health Administration

Epidemiology

Engineering

2. Reports for Institutions—Under direction of the Bureau of Institutional Health Administration

Make three copies

- (a) for institution submitting specimen
- (b) for Bureau of Institutional Health Administration
- (c) for our files

A copy of reports on all positive cases in institutions will be sent to the Bureau of Epidemiology

Institutions:

State Public School, Coldwater

Michigan Reformatory, Ionia

Michigan State Prison, Jackson

Branch Prison, Marquette

Industrial School for Boys, Lansing

Industrial Home for Girls, Adrian

II. Water Reports

Reports will contain the following information:

Town or township from which sample was collected

Name of person to whom report is made

Name of person collecting specimen, and date and time of collection

Source of specimen, private, municipal or railroad

Bacteriological findings

The reports will be given to the Bureau of Engineering to be mailed out. The original and first carbon copies will be given to them together with the original laboratory blank. Specimens which are not accompanied by information giving the source and location of the sample will be held until necessary information is

received. A blank will be sent to individuals submitting such specimens with a request that they be filled out and returned.

1. Bacteriological Examinations

(a) Railroad samples

Two copies

(1) for our files. (2) for engineers' files

(b) Lansing Routine

Four copies

(1) for our files. (2) for engineers' files. (3) for City Water Works, Lansing. (4) for City Health

(c) Samples from cities or persons

Three copies

(1) for our files. (2) for engineers' files. (3) for person submitting specimen

(d) Samples in which question of B. typhosus is involved

Four copies

(1) for our files. (2) for Bureau of Epidemiology. (3) for individual submitting specimen. (4) for engineers' files

2. Chemical Examinations

Three copies

(a) for our files. (b) for engineers' files. (c) for person submitting specimen

III. Smears for Gonorrhea

Intracellular gonococci (Gc)+, reported as "found"

Intracellular gonococci (Gc)-, reported as "not found"

Pus cells+, reported as "found"

Pus cells-, reported as "not found"

Extracellular gonococci+, reported as "extracellular gram negative diplococci found"

1. Negative reports

Two copies

(a) for our files. (b) for physician submitting specimen

2. Positive reports

Three copies

(a) for our files. (b) for Bureau of Epidemiology (to be sent in sealed envelope). (c) for physician submitting specimen

With all positive reports enclose a yellow venereal disease report blank and franked envelope.

IV. Wassermann and Kahn Tests

The full name and address of patient must be given before report

is mailed to physician. If omitted it must be written for and report held until necessary information is received. If case has been reported before, report may be made without address. Reports will be made only to the physician or institution submitting specimen. Patients may only get their reports from their physician. Phone all local reports.

1. Two, Three and Four plus reports

Three copies

- (a) for our files. (b) for physician submitting specimen. (c) for Bureau of Epidemiology (to be sent in sealed envelope)

2. All other reports

Two copies

- (a) for our files. (b) for physician submitting specimen

3. Exceptions:

Maternity Hospitals and Child Caring and Placing Agencies

Three copies

- (a) for our files
(b) for State Board of Corrections and Charities
(c) for institutions submitting specimen

With all strongly positive reports (++, +++, +++) a yellow venereal disease report blank and return franked envelope are to be sent.

V. Transudates and Exudates

1. Spinal fluids other than Wassermann

- (a) If Lange's colloidal gold reaction, report on Spinal Fluid blank
(b) If bacteriological examination, report on Miscellaneous Examination blank
(c) Make two copies
(1) for our files. (2) for physician submitting specimen

2. All body fluids not otherwise classified, swabs with pus, etc.

- (a) Report on Miscellaneous Examination blank
(b) Make two copies
(1) for our files. (2) for physician submitting specimen

VI. Milk Examinations

1. Bacteriological Examination

- (a) Report on Miscellaneous Examination blank
(b) Make two copies
(1) for our files. (2) for individual submitting specimen

Except in cases in which a question of typhoid is involved.
In such cases make three copies

- (1) for our files. (2) for individual submitting specimen. (3) for Bureau of Epidemiology

NOTE: Except in special cases, such as City of Lansing, all specimens of milk are referred to the Food and Drug Division, Department of Agriculture, through the Director of Laboratories.

2. Chemical Examination of Mother's milk

- (a) Report on Miscellaneous Examination blank
(b) Make two copies

- (1) for our files. (2) for physician submitting specimen

Specimens received from individuals with no physician's name will be referred to the Bureau of Child Hygiene and Public Health Nursing through the Director of Laboratories.

VII. Blood Examinations except Wassermann and Typhoid

1. Blood culture

- (a) Report on Miscellaneous Examination blank

2. Hematology

- (a) Report on Hematology blank

3. Chemical Analysis

- (a) Report on Chemical Examination of Blood blank

4. Two copies

- (a) for our files. (b) for physician submitting specimen

VIII. Throat Infections

1. Diagnosis

- (a) Diphtheria

Positive, reported as "B. diphtheria found."

Four copies

- (1) for our files. (2) for Bureau of Epidemiology.

- (3) for Health Officer of patient's city. (4) for physician submitting specimen

Wire all positive diagnoses giving history of case and charge to Antitoxin Division.

Negative, reported as "B. diphtheria not found."

Two copies

- (1) for our files. (2) for physician submitting specimen.

- (b) Streptococcus

I, II, III with per cents are "Hemolyticus found" omitting the per cent. IIIa is reported as such.

Four copies

- (1) for our files. (2) for Bureau of Epidemiology. (3) for Health Officer of patient's city. (4) for physician submitting specimen.

Green producing streptococci found is reported as such.

Variety of organisms found is reported as such.

Negative, reported as "not found."

Hemolytic streptococci negative is reported as "Hemolytic streptococci not found."

Two copies

(1) for our files. (2) for physician submitting specimen.

(c) Organisms of Vincent

Positive, reported as "found."

Negative, reported as "not found."

Two copies

(1) for our files. (2) for physician submitting specimen.

2. Release

(a) Diphtheria

Positive, reported as "B. diphtheria found."

Negative, reported as "B. diphtheria not found."

Three copies

(1) for our files. (2) for Health Officer of patient's city. (3) for physician submitting specimen.

(b) Streptococcus

Reported as in Streptococcus Diagnosis, omitting copy for Bureau of Epidemiology.

3. Carrier

Carriers will be reported as Diagnosis classification. A large group of carriers may be reported by letter, using this form:

On.....(Date).....we received.....
(Number).....throat swabs from you from the
Junior High School, which we have examined for suspected
diphtheria carriers.

(a) The cultures were all negative: "B. diphtheria not found."

(b) The cultures for Bernice May and Harold Wright were positive: "B. diphtheria found."

(1) We are running a virulence test on these cultures which we will report later.

(2) Please send confirmatory swabs for virulence tests. All other cultures were negative: B. diphtheria not found.

4. Virulence test.

Reports will be written from blue cards. "B. diphtheria found".....(date of inoculation). Virulence test:
.....(Result).....

5. General Considerations.

"No Growth" will be reported as such

"Contaminated" will be reported as "contaminated culture."

Reports for physicians with identical results, if stamped the same on top of the blue blank, may be reported on same blank. If colored crayon laboratory number indicates two swabs, report will be "two swabs examined."

Wire and telephone reports requested. Telephone all local reports. Diphtheria reports will be written with red type-writer ribbon.

6. Sunday diphtheria Reporting

(a) All releases will be written in the regular manner

(b) Diagnoses

(1) Positive diagnoses, "B. diphtheria found," will be wired or written in the regular manner.

(2) Negative diagnoses may be held over until Monday.

(c) Reports which are telephoned or wired will not be written until Monday.

(d) Phone all local reports. City health office not open on Sunday.

(e) Streptococci readings need not be reported until Monday.

IX. Typhoid Dysenteriae

1. Diagnosis

Positive

(a) Widal, reported as "positive"

(1) Microscopic

(2) Macroscopic

Give dilution, agglutination, and time as noted by bacteriologist under serum reaction.

(b) Feces, blood culture and urine, reported as "B. typhosus found" or "B. dysenteriae found," giving dilution, agglutination and time as noted by bacteriologist under serum reaction.

(c) Make four copies

(1) for our files. (2) for Bureau of Epidemiology. (3) for Health Officer of patient's city. (4) for physician submitting specimen.

Negative

(a) Widal, reported as "negative"

(b) Feces, blood culture and urine, reported as "Organisms of typhoid-dysentery group not found."

(c) Make three copies

(1) for our files. (2) for Bureau of Epidemiology. (3) for physician submitting specimen.

Treat Paratyphoid the same as Typhoid for Health Regulations.

2. Release

Reports are written in the same manner as in diagnoses.

Designate the fact that specimen is for release.

(a) Make four copies

(1) for our files. (2) for Bureau of Epidemiology. (3) for Health Officer of patient's city. (4) for physician submitting specimen.

X. Urine

(1) Chemical Examinations

(a) Report on Urine Analysis blanks.

(b) Charge \$1.00 for qualitative reports and send bill with report.

(c) Quantitative analyses are made free of charge.

(d) Make two copies

(1) for our files. (2) for physician submitting specimen.

2. Bacteriological Examinations

(a) Report on Miscellaneous Examination blanks

(b) Make two copies

(1) for our files. (2) for physician submitting specimen.

XI. Sputum—Examination for B. Tuberculosis

1. B. tuberculosis, reported as "found" or "not found."

2. Report "character," "cells," and "other organisms" as given.

3. Positive

Four copies

(1) for our files. (2) for Bureau of Epidemiology. (3) for Health Officer of patient's home. (4) for physician submitting specimen.

4. Negative

Two copies

(1) for our files. (2) for physician submitting specimen.

When individuals submit sputum without their physician's name, write for doctor's name. If they fail to answer or have no physician, send report to Health Officer of patient's city. Notify patient that report has been sent to Health Officer.

XII. Feces

1. Intestinal Parasites, Occult Blood, etc.

(a) Report on Miscellaneous Examination blank

(b) Make two copies

(1) for our files. (2) for physician submitting specimen.

2. B. typhosus. See Typhoid Dysenteriae Diagnosis and Release.

XIII. Unclassified Examinations and Toxicological Examinations are reported as indicated by examiner.

XIV. Animal Pathology was transferred to Department of Agriculture, October 1, 1923.

Method of Card File Entry

I. Recording examinations of

1. Throat, nasal and ear swab for B. diphtheria and streptococcus

2. Blood for Wassermann reaction and Kahn precipitation test

3. Pus smears for gonorrhea

4. Sputum for B. tuberculosis

5. Blood, urine and feces for typhoid

II. Entries made

1. Ten on each card, each recording

(a) number (b) date of receipt of specimen (c) patient's name (d) result of examination (e) date of report

2. Abbreviations used

(a) Throat, nasal and ear swabs for diphtheria and streptococcus

B. diphtheria found.

+D, Diagnosis. +R, Release. +C, Carrier.

B. diphtheria not found.

-D, Diagnosis. -R, Release. -C, Carrier.

-strep, Streptococcus not found, variety of organisms found.

Hem, Hemolytic streptococcus found.

-Hem, Hemolytic streptococcus not found.

Green, Green producing streptococcus found.

-V, Organisms of Vincent not found.

+V, Organisms of Vincent found.

(b) Blood for Wassermann reaction and Kahn precipitation test.

+(as indicated), Wassermann test positive.

-, Wassermann test negative.

ppt.+ (as indicated), Kahn precipitation test positive.

ppt.-, Kahn precipitation test negative.

- (c) Pus smears for gonococci
 — (give source), Intracellular diplococci not found.
 + (give source), Intracellular diplococci found.
 extra (give source), Extracellular diplococci found.
 U = urethra; C = cervix; V = vagina; BD = bartholin duct;
 eye, rectum, urine, as such.
- (d) Sputum for *B. tuberculosis*
 —, *B. tuberculosis* not found.
 +, *B. tuberculosis* found.
 Pneu, *Pneumococcus* found.
 Cult, no organisms of significance found on culture.
 Same as in (a) for *Streptococcus*.
- (e) Blood, urine, feces for typhoid
B. typhosus found.
 +F, Feces. +BC, Blood culture. +U, Urine.
B. typhosus not found.
 —F, Feces. —BC, Blood culture. —U, Urine.
 +Wid, Widal positive.
 —Wid, Widal negative.
- (f) Blood, urine, feces for Paratyphoid.
Paratyphosus "A" found.
 Para A F, Feces. Para A BC, Blood culture. Para
 A U, Urine.
Paratyphosus "B" found.
 Para B F, Feces. Para B BC, Blood culture. Para
 B U, Urine.
 Same as in (a) for *Streptococcus* (indicate kind of specimen).
- (g) Feces for *Dysenteriae*.
 +Dys, *B. dysenteriae* found.
 —Dys, *B. dysenteriae* not found.

3. Location

- (a) Card file is in steel files in record room.
 (b) Original blanks are in vault, filed chronologically.

CHAPTER VII

OUTLINE OF PROCEDURE OF WASSERMANN AND KAHN PRECIPITATION TESTS

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Preparation of Serums for Both Tests

- I. Numbering of Blood Specimens.
 1. When blood specimens are received from the mail entry room at about 9:00 and 11:00 A. M., place them in racks in order and number each to correspond with the number on the accompanying blank. Include in the daily total the number of specimens received from noon to noon.
 2. Initial each blank after determining that serologic examination for syphilis is requested.
- II. Preparation for Centrifugation.
 1. Remove corks from vials and split tops off from the Keidal tubes.
 2. Break up the clots and, when possible, remove with wooden applicators.
- III. Centrifugation.
 1. Centrifuge the specimens for 20 minutes at 1000 r.p.m.
- IV. Separation of Serums from Clots.
 1. After centrifuging, place the specimens in racks in order of numbers, with a row of empty vials, bearing corresponding numbers, back of each row of specimens.
 2. Pipette the serum from each specimen into the corresponding empty vial by means of a bulb-capillary pipette.
 3. Rinse the pipette in physiologic salt solution before pipetting each specimen, using two 500 cc. beakers of physiologic salt solution.
 - (a) Fill the pipette from the first beaker and discard salt solution three times.
 - (b) Rinse the pipette in the second beaker of salt solution.
 - (c) Discard the salt solution in the two beakers and add fresh each morning and again after about 100 specimens have been pipetted.
 - (d) If serum is drawn up into the bulb of a pipette, discard that particular pipette and substitute a clean one.
- V. Inactivation.
 1. Heat the serums for 30 minutes at 56° C. in a water bath previous to their use in the tests.

Procedure of Wassermann Test

- I. Reagents. The test is performed with patient's serum, antigen, salt solution and a hemolytic system of sheep cells, guinea pig complement and anti-sheep cell amboceptor. (Keep antigen and salt solution at room temperature and all the other reagents in the ice box between operations.)
 1. Serum. Prepare patient's serum as described under "Preparation of Serums for Both Tests."
 2. Antigen.
 - (a) Preparation. Prepare an alcoholic extract of powdered beef heart muscle as described later under "Standard Antigen for Kahn Test."
 - (b) Cholesterinization. Add 2 mg. cholesterin per cc. of alcoholic extract.
 - (c) Titration and Standardization.
 - (1) Dilute the 0.2 per cent cholesterinized antigen with physiologic salt solution in the proportions: 1:50, 1:100, 1:150, 1:200, 1:250, respectively. Make each dilution by adding the salt solution from a 10 cc. pipette to the antigen, at first drop by drop, gently rotating the flask. After 5 cc. of salt solution has been added drop by drop, add the remainder more rapidly—1 cc. at a time until half of the salt solution has been added and then 5 cc. at a time. The final mixture should be somewhat opalescent and possess slight turbidity.
 - (2) Dilute each of 2 four plus Wassermann serums with physiologic salt solution in the proportions: 1:2, 1:4, 1:8. Use these 6 serums together with 2 serums which have given three plus, one which has given two plus and one which has given a negative reaction in the daily tests in the titration—testing each serum with each of the antigen dilutions.
 - (3) Pipette 0.01 cc. of each specimen into each of 5 tubes, making 5 series of 10 tubes.
 - (4) Into each tube of the first series pipette 0.1 cc. antigen dilution 1:50, in the second series 1:100, and in the third, fourth and fifth series, antigen dilutions 1:150, 1:200 and 1:250, respectively.
 - (5) Add two units (0.1 cc.) complement to each of the 50 tubes.
 - (6) Add 3 drops of salt solution to each tube.
 - (7) Set up 5 tests to determine the anticomplementary properties of the antigen, using 0.4 cc. of antigen dilu-

tions 1:50, 1:100, 1:150, 1:200 and 1:250, respectively, with 0.1 cc. (2 units) complement.

- (8) Set up 5 tests to determine whether the antigen is hemolytic, using 0.4 cc. of antigen dilutions 1:50, 1:100, 1:150, 1:200 and 1:250, respectively.
- (9) Place all 60 tubes of the titration in the ice box for a fixation period of 2 hours.
- (10) After the fixation period add 0.1 cc. standard sheep cell suspension and 0.1 cc. (2 units) amboceptor to each tube.
- (11) Place the tubes in the 37° C. water bath for 15 minutes.
- (12) Record the relative completeness of complement fixation in each tube as —, ±, +, ++, +++ or ++++ as indicated by the degree of hemolysis. Check the readings after the cells have been allowed to settle.
- (13) Repeat the complete titration on at least 3 different days and determine the final titer (the optimum antigen dilution) by comparing the results of the several titrations. Based on the findings in Table 1, the titer is 1:150.

TABLE 1—TYPICAL WASSERMANN ANTIGEN TITRATION

Serum No.	1	2	3	4	5	6	7	8	9	10	Anticomplementary Control	Hemolytic Control
Serum 0.01 cc.	++++ (diluted)			++++ (diluted)								
	1:2	1:4	1:8	1:2	1:4	1:8	+++	+++	++	-		
Dilution of Antigen.	Fixation of Complement.										Hemolysis	
1:50.....	++++	++++	+++	++++	++	+	++++	++++	±	-	Delayed	None
1:100.....	++++	++++	+++	++++	+	±	++++	++++	-	-	Complete	None
1:150.....	++++	++++	+++	++++	+	±	++++	++++	-	-	Complete	None
1:200.....	++++	+++	++	+++	+	±	++	+	-	-	Complete	None
1:250.....	++++	+++	+	+	+	±	+	+	-	-	Complete	None

3. Salt Solution. Prepare physiologic salt solution in the usual manner.

4. Complement.

- (a) Obtain complement by bleeding guinea pigs, under anesthesia, directly from the heart, using a 10 cc. Luer syringe and a 20 gauge needle. Bleed no less than three pigs for a series of tests.

- (b) Transfer the blood from each pig to a centrifuge tube, slant the tube and allow the blood to clot.
- (c) Break up the clots and place the blood in the ice box for several hours.
- (d) Remove the serum complement from the clots, centrifuge and remove the supernatant clear serum for titration as described under "Wassermann technic."

5. Amboceptor.

- (a) Select young, healthy rabbits.
- (b) Prepare sheep cells as follows:
 - (1) For 1 cc. of packed cells, use about 3 cc. of sterile blood.
 - (2) Add physiologic salt solution to make 15 cc.
 - (3) Filter cell suspension through sterile cheese cloth to remove fine clots.
 - (4) Centrifuge for 5 minutes, remove the supernatant fluid, and add fresh salt solution to make 15 cc.
 - (5) Repeat (4) until cells have been washed 5 times.
 - (6) Make a 20 per cent cell suspension by adding 4 cc. of sterile salt solution to each cc. of packed cells.
- (c) Inoculate the rabbit intravenously (marginal ear vein).
 - (1) Give 2, 2, and 3 cc., respectively, of a freshly prepared 20 per cent suspension of cells on 3 consecutive days.
 - (2) Allow the rabbit to rest for 3 days.
 - (3) Give a series of 3, 4 and 5 cc., respectively, of 20 per cent cell suspension on 3 consecutive days.
 - (4) Make a test bleeding after 5 days. If the hemolytic titer is high, bleed the rabbit from the carotid artery. If the titer is too low, give another series of inoculations.
- (d) Determine the unit of new amboceptor by preparing a series of dilutions of amboceptor serum and titrating each with 0.1 cc. of a 5 per cent suspension of sheep-cells and 0.1 cc. (2 units) of diluted pooled guinea pig complement.
 - (1) Make a preliminary series of dilutions: 1:100, 1:1000, 1:2000, 1:4000 as follows:

Amboceptor Serum		Salt Solution		Dilution of Amboceptor
0.5 cc. undiluted	+	4.5 cc.	=	1:10
0.5 cc. 1:10	+	4.5 cc.	=	1:100
0.5 cc. 1:100	+	4.5 cc.	=	1:1000
2.0 cc. 1:1000	+	2.0 cc.	=	1:2000
2.0 cc. 1:2000	+	2.0 cc.	=	1:4000

- (2) Titrate each dilution, using the quantities of reagents outlined in Table 2.

TABLE 2—SET-UP FOR TITRATION OF AMBOCEPTOR

Tube.....	1	2	3	4	5	6	7	8	9	10
Amboceptor (cc.).....	0.1	0.09	0.08	0.07	0.06	0.05	0.04	0.03	0.02	0.01
Complement (cc.) (2 units).....	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Sheep Cells (cc.) (5 per cent).....	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Saline (drops).....	2	2	2	3	3	3	3	4	4	4

- (3) Read the unit (the least amount of amboceptor which produces hemolysis of 0.1 cc. of a 5 per cent suspension of sheep cells in the presence of 0.1 cc.—2 units—of complement) in each case after 15 minutes' incubation in the water bath at 37° C. The unit desired is 0.05 cc., so that 0.1 cc. will contain 2 units of amboceptor.
- (4) If that amboceptor serum dilution in which the unit is 0.05 lies, for example, between 1:2000 and 1:4000, prepare a series of dilutions 1:2000, 1:2400, 1:2800, 1:3200, 1:3600, 1:4000 and titrate each, as outlined above.
- (5) After finding the dilution of amboceptor serum which gives a unit of 0.05 cc., repeat the titrations daily for a week with different pooled complements in order to establish a permanent amboceptor unit. If the titer is, for example, 1:2000, make a stock solution in a dilution of 1:100. For the daily tests dilute 1 cc. of this with 19 cc. of salt solution. Check this dilution by a daily amboceptor titration as described under "Daily Set-up of Tests."

6. Sheep Cells.

- (a) Obtain blood once every five days from the carotid artery of a sheep.
- (b) Prepare standard suspension of cells for the daily tests as described under "Wassermann Technic."

II. Wassermann Technic

1. Daily Titration of Complement. Make complement titrations the first thing in the morning.
 - (a) Dilute each serum and the pooled serum 1:10 with physiologic salt solution.
 - (b) Measure 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1 cc. quantities, respectively, into 10 tubes.
 - (c) Add 0.1 cc. amboceptor (2 units) and 0.1 cc. sheep cells (5 per cent suspension) to each quantity of complement.

(d) Use two control tubes for each complement titration.

- (1) Into the first control tube measure 0.1 cc. of the diluted serum, 0.4 cc. of antigen diluted for the tests and 0.1 cc. each of cells and amboceptor.
- (2) Into the second control tube measure 0.1 cc. complement and 0.1 cc. cells but no amboceptor.
- (3) Make the volumes in all tubes uniform by the addition of salt solution.
- (4) Place all tubes in water bath at 37° C. for 15 minutes.
- (5) Observe degree of hemolysis in all tubes. There should be no delay in hemolysis in first control and no hemolysis in second. Table 3 shows typical complement titration.

TABLE 3—TITRATION OF COMPLEMENT

Tube.....	1	2	3	4	5	6	7	8	9	10
Complement (cc.) (1:10).....	0.1	0.09	0.08	0.07	0.06	0.05	0.04	0.03	0.02	0.01
Amboceptor (cc.) (2 units).....	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Sheep Cells (cc.) (5 per cent).....	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Saline (drops).....	2	2	2	3	3	3	3	4	4	4

Control Tubes.....	1	2
Complement (cc.) (2 units).....	0.1	0.1
Antigen.....	0.4	
Amboceptor (cc.) (2 units).....	0.1	
Sheep Cells (cc.) (5 per cent).....	0.1	0.1
Saline (drops).....		2

- (6) Dilute complement for the tests, based on results of the titration. The unit (the smallest amount of complement producing hemolysis of 0.1 cc. of 5 per cent sheep cell suspension in the presence of 0.1 cc. or 2 units amboceptor) desired is 0.05 cc. so that 0.1 cc. will contain 2 units. If 0.04 cc. is the smallest amount producing complete hemolysis, dilute the serum complement 1:12.5, instead of 1:10, in accordance with the following equation:

$$(1:10) : (1:x) = 0.04 : 0.05$$

or

$$10 : x = .04 : .05$$

$$x = 12.5$$

Use the results obtained with the pooled serum largely as the criterion for the dilution of complement. Discard any individual complement which has a hemolytic titer less than 0.06 cc.

Employ the following scheme in making complement dilutions:

Titration Unit	Dilution for Tests
0.06	1+7
0.05	1+9
0.04	1+11.5
0.03	1+15

2. Dilution of Antigen for Tests. Using the antigen titer previously determined, mix the antigen with the required amount of salt solution in the usual manner. Prepare about 30 cc. of antigen mixture per 100 tests.
3. Standard Sheep-Cell Suspension. Prepare about 50 cc. cell suspension for each 100 tests.
 - (a) For each cc. of packed sheep cells required for the suspension, place about 3 cc. blood in a centrifuge tube and add physiologic salt solution to make 15 cc.
 - (b) Mix the cells with the salt solution, centrifuge for 5 minutes, remove supernatant fluid with a bulb pipette, and make up the volume with salt solution.
 - (c) Repeat (b) until the cells have been washed 4 times. In the fourth washing, use a centrifugation period of 15 minutes to pack the cells.
 - (d) Make a 5 per cent cell suspension by adding 19 cc. of salt solution to each cc. of packed cells.
 - (e) Filter through several layers of cheese cloth.
4. Re-Inactivation of Serum. Since the Wassermann test is performed on serums which were tested by the Kahn test the previous day, the specimens stand in the ice box over night after their inactivation period. Just before they are pipetted for the Wassermann test, re-inactivate the serums for 5 minutes at 56° C.
5. Set-Up of Tests
 - (a) Pipetting of Serums. Set up three tubes for each specimen using 0.02, 0.01 and 0.02 cc., respectively, of serum. The third tube is the serum control.
 - (b) Pipetting of Antigen. Add diluted antigen to the first two serum tubes in 0.1 cc. quantities. Mix the antigen with complement, before adding, as explained under (c).
 - (c) Pipetting of Complement. Add complement, diluted according to titration, to all tubes in 0.1 cc. quantities. In the case of tubes 1 and 2 which receive both complement and antigen, mix these two ingredients in equal amounts immediately before using and add 0.2 cc. of the mixture.
 - (d) Addition of Salt Solution. Add 2 drops of salt solution to

tubes 1 and 2 and 3 drops to tube 3 to make each volume approximately 0.3 cc.

- (e) Mixing of Ingredients. Shake each rack of tests vigorously to thoroughly mix the ingredients.

6. Controls. Include the following controls with each series of tests.

- (a) Daily antigen titration.

- (1) To each of three tubes (1, 2 and 3) add 0.01 cc. syphilitic (+ + + +) serum.
- (2) Add diluted antigen in 0.1, 0.05 and 0.025 cc. quantities, respectively.
- (3) Add two units (0.1 cc.) of complement to each tube.
- (4) Make up the volume by adding 3 drops of salt solution to each tube.

- (b) Anticomplementary control of antigen.

- (1) To three tubes (4, 5 and 6) add 0.4, 0.2 and 0.1 cc quantities of diluted antigen, respectively.
- (2) Add two units (0.1 cc.) of complement to each tube.
- (3) Make the volumes uniform by the addition of salt solution.

- (c) Hemolytic Control of Antigen. To another tube (7) add 0.4 cc. diluted antigen.

- (d) Set up regular tests with three serums which give a + + + +, $\pm$ and -, respectively, on the previous day.

- (e) Include serum controls of all specimens as noted under 5 (a).

7. Fixation Period. Immediately after addition of salt solution and mixing of the ingredients (about 9:30 A. M.) place the tests and controls in the ice box (about 5° C.) and leave for 2 hours.

8. Daily Amboceptor Titration.

- (a) During the fixation period, titrate the amboceptor, employing the quantities of reagents indicated in Table 2 under "Amboceptor."

- (b) If the unit is not 0.05, make proper dilution according to the proportion: Dilution used : Dilution required = Actual unit : Desired unit (.05.)

ILLUSTRATION

Amboceptor Stock Dilution = 1:100

Preliminary dilution for titration and use in tests	=	1:20 dilution of stock dilution (1:2000)
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Quantity of preliminary dilution prepared for 200 tests	=	80 cc. (4 cc. of 1:100 + 76 cc. salt solution)
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Desired amboceptor unit	=	0.05 cc.
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Example 1.

Unit determined by titration = 0.06

Dil. used : Dil. required = Actual unit : Desired unit

Then 2000 : x = 0.06 : 0.05

$$6x = 10000$$

$$x = 1666\frac{2}{3}$$

Amboceptor dilution to be made for tests = 1:1600

Amount Stock dil. (1:100) required for 80 cc. (1:1600) = 5 cc.

Amount Stock dil. already in 80 cc. (1:2000) = 4 cc.

Amount Stock dil. to be added to 80 cc. = 1 cc.

Example 2.

Unit determined by titration = 0.04

Then 2000 : x = .04 : .05

$$4x = 10000$$

$$x = 2500$$

Amboceptor dilution to be made for tests = 1:2500

Dilution of stock dil. (1:100) necessary to make 1:2500 dil. of amboceptor = 1:25 or 1 part stock dil. + 24 parts salt solution.

Amount Stock dil. required for 80 cc. of 1:2500 = 3.2 cc.

Amount Stock dil. already in 80 cc. of 1:2000 = 4.0 cc.

Excess Stock dil. already in 80 cc. of 1:2000 = .8 cc.

Salt solution to be added to 80 cc. (1:2000) for dilution of excess stock dilution = .8x24 = 19.2 cc.

9. Addition of Cells and Amboceptor.

- After the 2 hour fixation period (at about 11:30 A. M.), remove the tests from the ice box.
- Make a mixture of equal parts of 5 per cent sheep cell suspension and amboceptor dilution—mixing the two ingredients rapidly.
- Add 0.2 cc. of the mixture to each tube.

10. Incubation Period.

- After addition of the amboceptor-cell mixture, shake the tests to thoroughly mix the ingredients.
- Place tests in water bath at 37° C. for 15 min.—or until the serum control tubes show complete hemolysis.

11. Reading of Results.

- Read the tests immediately after they are removed from the water bath (about 11:45 A.M.) and again after they have stood for an hour and a half at room temperature (about 1:15 P. M.). Average the first and second readings for the final result.

- (b) For each serum, record the degree of complement fixation on the basis of $-$, $\pm$, $+$, $++$, $+++$, $++++$ as indicated by the relative completeness of the hemolysis of red cells. For example, if the hemolysis is complete in both tubes of the test as well as in the 3rd tube (control), no fixation of complement is indicated. If there is no hemolysis in both tubes of the test, with complete hemolysis in the control, the complement fixation is indicated as four plus $++++$. Table 4 shows the daily set-up of tests and Table 5 outlines a scheme for reading and interpreting the results.

12. Recording Results. While one worker reads the tests, another records the findings on daily report sheets which become the original record of all Wassermann results. Record the first and second readings in pencil and the average or final in ink.

TABLE 4—DAILY SET-UP OF WASSERMANN TESTS

	Test with each patient's serum and with $++++$, $\pm$ and $-$ serum			Antigen controls with each series of tests.						
				Titration with $++++$ serum			Anticomplementary			Hemolytic
	1	Tubes 2 3 (control)		1	Tubes 2 3		4	Tubes. 5 6		Tube 7
Serum (cc.).....	.02	.01	.02	.01	.01	.01	..	..	..	..
Antigen (cc.).....	.1	.1	..	.1	.05	.025	.4	.2	.1	.4
Complement (cc.) (2 units).	.1	.1	.1	.1	.1	.1	.1	.1	.1	..
S alt Sol. (drops).....	2	2	3	3	3	3	..	2	3	..
2 Hr. Fixation in Ice Box (6° C.)										
Amboceptor.....	.1	.1	.1	.1	.1	.1	.1	.1	.1	.1
Sheep Cells (5% suspension)	.1	.1	.1	.1	.1	.1	.1	.1	.1	.1
15 Min. Incubation in Water Bath (37° C.)										
Results.	See Table 5.			Hemolysis (C = Complete; 0 = None)						
				0	0	0	C	C	C	0

TABLE 5—SCHEME FOR READING WASSERMANN RESULTS

Tube No. (of each Test)	1	2	3' (control)	Results
Serum Amounts cc.....	.02	.01	.02	
	Hemolysis			Complement Fixation
Some typical findings	none none none slight considerable complete none	none slight considerable considerable complete complete none	complete complete complete complete complete complete none	++++ ++++ ++ + ± — Specimen Anticomplementary

II. Wassermann Test with Spinal Fluids.

1. Set-Up of Tests.

- Measure 0.4, 0.3, 0.2 and 0.1 cc., respectively, spinal fluid into 4 tubes.
- Use all other reagents in same quantities as in test with serum.
- For a control of the anticomplementary properties of each fluid, test 0.4 cc. without the addition of antigen.

2. Interpretation of Results.

- If fixation of complement is complete in all 4 tubes of the test, read the reaction four plus; if complete with only the three larger quantities, three plus; if complete with only the two largest quantities, two plus; if complete with only the 0.4 cc. quantity, one plus; if nearly complete with the 0.4 cc. quantity and partial with the second quantity, doubtful.
- Report any specimen showing fixation in the control tube "anti-complementary."

REFERENCES: The Wassermann Test and Its Interpretation.—R. L. Kahn. *J. Lab. and Clin. Med.*, VI (July), 579, 1921; Pathogenic Microorganisms.—Park and Williams. Lea & Febiger, Philadelphia, 1924; Infection, Immunity and Biologic Therapy.—J. A. Kolmer. Saunders, Philadelphia, 1924.

Procedure of Kahn Precipitation Test

I. Routine Test with Serum.

A. Reagents. The test is performed with patient's serum, antigen and physiologic salt solution.

- Serum. Prepare patient's serum as described under "Preparation of Serums for Both Tests."
- Standard Antigen.

(a) Extraction of Powdered Beef Heart with Ether.

Extraction 1.

- Place 25 gm. of Bacto Beef Heart in a 250 Erlenmeyer flask.

- (2) Add 100 cc. ether (anesthesia)
- (3) Shake the flask from time to time during an interval of ten minutes.
- (4) Filter through filter paper, applying gentle pressure to the beef heart by means of a spatula. When no more drops of ether pass through the funnel, filtration is complete.

Extraction 2.

- (1) Return the moist beef heart to the original extraction flask.
- (2) Add 75 cc. ether.
- (3) Shake for a ten minute interval.
- (4) Filter as in Extraction 1.

Extraction 3.

- (1) Transfer the beef heart to the flask for a third time.
- (2) Add 75 cc. ether.
- (3) Shake the mixture for a ten minute interval.
- (4) Filter as in Extraction 1.

Extraction 4.

- (1) For the fourth and last time transfer the moist beef heart to the flask.
- (2) Add 75 cc. ether.
- (3) Shake for ten minutes.
- (4) Filter, using a fresh filter paper.
- (5) Transfer the moist powder to a sheet of white paper and dry until no ether odor is detectable.

(b) Preparation of Alcoholic Extract.

- (1) Weigh the dried powdered muscle and place in the ether extraction flask which has been dried and freed of ether odor.
- (2) Add 5 cc. of 95 per cent alcohol per gram of powder.
- (3) Shake the mixture for ten minutes.
- (4) Leave at room temperature (21° C.) for three days undisturbed.
- (5) At the end of this 3 day period, shake the mixture for five minutes.
- (6) Filter through filter paper until clear.
- (7) Store the filtrate in the dark at room temperature as stock antigen solution. (Use tin-foil covered corks as stoppers for flasks used in antigen preparation and storing.)

(c) Cholesterinization of Alcoholic Extract.

- (1) Add 6 mg. of chemically pure cholesterin per cc. of alcoholic extract.
- (2) Dissolve the cholesterin by rotating the flask in a water bath at 37° C.
- (3) When all the cholesterin has been dissolved, filter the antigen.
- (4) Allow to stand at least one day before titration.

(d) Titration of Antigen.

- (1) Add 1 c. c. of cholesterinized antigen to each of five standard antigen dilution tubes. (Fig. 13b Appendix D.)
- (2) To five similar tubes add 0.8, 0.9, 1.0, 1.1 and 1.2 c.c., respectively, of physiologic salt solution.
- (3) Empty each salt solution tube into a given antigen tube and immediately pour the mixture back and forth at least six times to permit thorough mixing.
- (4) Test the solubility in salt solution of each of the five antigen dilution precipitates as follows:
 - a. Pipette 0.05, 0.025 and 0.0125 c.c. amounts, respectively, of each antigen dilution into 3 standard tubes for the test, using a 0.2 c.c. pipette graduated in 0.001 c.c. (Fig. 13d Appendix D). Deliver the quantities to the bottom of the tubes.
 - b. Add 0.15 c.c. of physiologic salt solution to each tube.
 - c. Shake all tubes vigorously for three minutes.
 - d. Add 0.5 c.c. salt solution to each tube.
 - e. Examine each tube to determine whether the original antigen dilution precipitate has gone back into solution. The antigen-salt solution mixture containing the smallest amount of salt solution in proportion to antigen, having a precipitate which goes back into solution in salt solution represents the end-point of this titration and determines the dilution of antigen for the tests. Table 6 gives an outline of a typical antigen titration.

TABLE 6—TYPICAL ANTIGEN TITRATION FOR ROUTINE TEST WITH SERUM

Antigen Dilution Series	1	2	3	4	5
Antigen+Salt Solution c.c.	1+.8	1+.9	1+1.0	1+1.1	1+1.2
Result of Dilution	Heavy precipitate in each Antigen dilution				
Scheme Used in Testing Solubility of Precipitate in Each Antigen Dilution	Tube No.	1	2	3	
	*Antigen Dilution c.c.	.05	.025	.0125	
	Salt Solution c.c.	.15	.15	.15	
	Tubes are shaken 3 minutes and 0.5 c.c. salt solution added to each. All are observed for precipitates.				
Solubility of Precipitate as De- termined by Three-Tube Test	Precipitate Not Soluble	Precipitate Not Soluble	Precipitate Soluble	Precipitate Soluble	Precipitate Soluble
Standard Antigen Dilution			Antigen + min- imum amount of salt solution giving precip- itate which dis- solves in salt solution		

3. Physiologic Salt Solution.

Prepare as for the Wassermann test.

B. Technic of Kahn Routine Test with Serum. The routine test consists of three tubes containing three different proportions of serum and antigen dilution in accordance with the following outline:

Tube No.	1	2	3
Serum: Antigen Dilution	3:1	6:1	12:1
Antigen Dilution c.c.	.05	.025	.0125
Serum c.c.	.15	.15	.15

(For glassware and apparatus for the test see Fig. 12 and 13.)

1. Preparation of Standard Antigen Dilution. Prepare antigen dilution for 40 tests at a time.

- If the antigen titer is 1+1, measure 2.5 c.c. of antigen into one antigen dilution tube and 2.5 c.c. of physiologic salt solution into another.
- Pour the salt solution into the antigen and immediately pour the mixture back and forth at least six times.
- Allow this antigen dilution to stand ten minutes at room temperature before pipetting.

2. Pipetting of Antigen Dilution. Pipette antigen dilution for 20 tests at a time immediately before the serum is to be added. Mix the antigen dilution frequently during the pipetting period. Deliver to the bottom of the tubes.

- Pipette the twenty 0.05 cc. amounts first, using a 1 cc. pipette on which 0.05 cc. graduations are indicated with a wax pencil.
- Pipette the 0.025 cc. amounts employing a 0.2 cc. pipette, properly marked with a wax pencil.

*Each antigen dilution is allowed to stand 30 minutes after mixing antigen and salt solution before solubility test[†] is made.

(c) Pipette the 0.0125 cc. amounts employing a marked 0.2 cc. pipette.

3. Pipetting of Serum.

- (a) Add 0.15 cc. of each serum to each of the 3 antigen dilution quantities by means of a 1 cc. pipette graduated in 0.01 cc.
- (b) As soon as the serums have been added for ten tests, shake the rack sufficiently to insure thorough mixing of the serum with antigen dilution.



Figure 1.—Reading of Results.

4. Control System.

- (a) Antigen Control. When pipetting antigen dilution for a series of tests, add 0.15 salt solution to each tube of the last set-up of three regular antigen amounts and include with the regular tests. All three tubes should show no precipitate.
- (b) Serum Controls. In the case of each positive reaction,

examine the serum used in the test for particles that might give the appearance of a specific precipitate.

- (c) Positive and Negative Controls. Include tests with at least one positive and one negative serum in each series of tests.
5. Shaking of Tests. After the serums have been mixed with antigen dilution, shake the mixtures for 3 minutes in a shaking machine (Fig. 12 Appendix D).

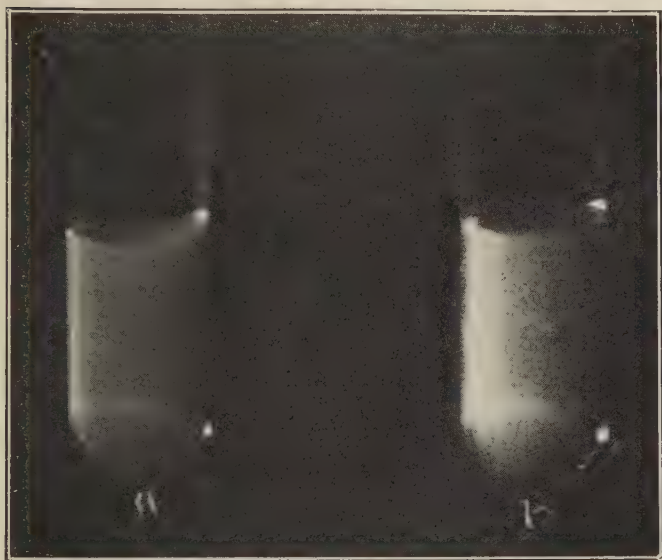


Figure 2.—Interpretation of Results.
A—No precipitation. Negative Reaction.
B—Marked precipitation. Positive Reaction.

6. Incubation Period. After the shaking period, place the tests in the 37° C. water bath for 15 minutes.
7. Addition of Salt Solution. After the 15 minute incubation period, add salt solution to each tube—1 cc. in the front row containing 0.05 cc. quantities of antigen dilution and 0.5 cc. in the other two rows.
8. Reading of Results. About five minutes after the addition of salt solution, read the tests in front of a window with a darkened background.
 - (a) Read the negative and strongly positive reactions without lifting the tubes from the racks.
 - (b) In the case of weak reactions, examine each tube individually. Lift the tube above the eye level, slant to spread the fluid into a thin layer and examine for a precipitate.

9. Interpretation of Results.

- (a) Interpret a definite precipitate suspended in a clear medium as four plus and proportionally weaker reactions as three, two and one plus, respectively.
- (b) For the final result to be reported, average the findings of the three tubes. A number of typical reactions are illustrated in Table 7.

TABLE 7—INTERPRETATION OF RESULTS OF ROUTINE TEST WITH SERUM

Tube No		1	2	3	Average of Reaction in the Three Tubes = Final Result
Serum: Antigen Dilution Antigen Dilution c.c. Serum Undiluted c.c.		3:1 .05 .15	6:1 .025 .15	12:1 .0125 .15	
Some Typical Reactions	Reaction No. 1 2	++++ ++++	++++ ++++	++++ ++++	++++
	3 4 5	++ + —	++++ ++++ ++++	++++ ++++ ++++	+++
	6 7 8	— — —	+++ ++ +	++++ ++++ ++++	++
	9 10 11	— — —	— — +	++++ ++++ ++	+
	12 13 14	— — —	± — ±	++ ++ +	±

10. Recording of Results. While one worker reads the results, another records the findings in all tubes on daily report sheets which become the original record of Kahn results.

II. Technic of Kahn Procedure for Spinal Fluids. The test with spinal fluids is based upon the concentration of the reacting substances in the fluid with ammonium sulfate. The concentrated fluid is tested with two different antigens, standard and special. Preparation of special antigen is described under Technic of Presumptive Procedure. On account of the use of ammonium sulfate it is necessary to make a special titration of antigen.

1. Titration of Standard Antigen for Spinal Fluid Procedure.

- (a) Measure five 1 c.c. quantities of standard antigen into five standard antigen dilution tubes.
- (b) Into five similar tubes measure 1.1, 1.2, 1.3, 1.4 and 1.5 c.c. physiologic salt solution, respectively.
- (c) Pour each of the saline amounts into an antigen tube and immediately pour the mixture back and forth at least six times.
- (d) Allow these mixtures to stand 20 minutes.

(e) Test the solubility of the precipitates in each with an ammonium sulfate solution, prepared by mixing 0.5 c. c. of saturated solution with 9.5 c.c. salt solution.

- (1) Pipette, in duplicate, 0.01 c.c. amounts of the five antigen dilutions, respectively, to the bottom of the tubes, using a 0.2 c.c. pipette graduated in 0.001.
- (2) Add 0.15 c.c. of the ammonium sulfate solution to each tube.
- (3) Shake the tubes for three minutes.
- (4) Add 0.5 c.c. salt solution.
- (5) Examine tubes for precipitates and determine antigen titer. The dilution of antigen and saline which contains the smallest amount of saline in proportion to antigen, giving a precipitate which is soluble in the ammonium sulfate solution represents the titer of the antigen for spinal fluids. A typical titration is given in Table 8.

TABLE 8—TYPICAL ANTIGEN TITRATION FOR TEST WITH SPINAL FLUID
(STANDARD ANTIGEN)

	Antigen Dilution Series				
	1	2	3	4	5
Antigen+salt solution, cc....	1+1.1	1+1.2	1+1.3	1+1.4	1+1.5
Result of dilution.....	Heavy precipitate in each antigen dilution.				
Scheme used in testing solubility of precipitate in each antigen dilution	Tube number.....		1	2	
	*Antigen solution, cc.....		0.01	0.01	
	5 per cent solution ammonium sulfate, cc.		0.15	0.15	
	Tubes are shaken 3 minutes and 0.5 cc. salt solution added to each. All are observed for precipitates.				
Solubility of precipitate	Precipitate not soluble	Precipitate not soluble	Precipitate not soluble	Precipitate soluble	Precipitate soluble
Standard antigen dilution				Antigen + minimum amount of normal saline giving precipitate which dissolves in 5 per cent ammonium sulfate solution.	

2. Titration of Special Antigen for Spinal Fluid Procedure. Make this titration just as the one for standard antigen except that the test mixtures of special antigen and salt solution are 1+1.4, 1+1.5, 1+1.6, 1+1.7, and 1+1.8, respectively.

3. Concentration of Spinal Fluid with Ammonium Sulfate.

- (a) Centrifuge each fluid.
- (b) Mix 3 c.c. clear supernatant fluid with 2 c.c. saturated ammonium sulfate solution in 15 cc. centrifuge tube.

*Each antigen dilution is allowed to stand 20 minutes after mixing antigen and salt solution before solubility test is made.

- (c) Allow to stand for 15 minutes in water bath at 56° C.
 - (d) Centrifuge for fifteen minutes to throw down the globulins.
 - (e) Pipette off supernatant fluid completely.
 - (f) Add 0.3 c.c. physiologic salt solution (1-10 original amount of spinal fluid used) to the globulin precipitate. Lower the salt solution into the center of the tube to avoid washing down ammonium sulfate from the inner wall of the tube. Use this globulin solution (concentrated fluid) in the test.
4. Set-up of Tests.
- (a) Preparation of Antigen Dilution.
 - (1) Dilute each antigen (standard and special) with salt solution as described under the test with serum, using the proportion indicated by the special titrations with ammonium sulfate.
 - (2) Allow the antigen dilution to stand for ten minutes before pipetting.
 - (b) Pipetting of Antigen Dilution. For each fluid to be tested, pipette 0.01 c.c. of each antigen dilution to the bottom of a tube, using 0.2 c.c pipette.
 - (c) Pipetting of Concentrated Fluid. Add concentrated spinal fluid in 0.15 c.c. quantities to the two tubes immediately after the antigen dilution has been pipetted.
 - (d) Controls.
 - (1) Into two tubes measure 0.01 c.c. of standard and special antigen dilution, respectively, and add to each tube 0.15 c.c. of an ammonium sulfate solution prepared by mixing 0.5 c.c. saturated ammonium sulfate with 9.5 c.c. saline. Include these tubes with the regular tests.
 - (2) Include positive and negative fluid controls with each series.
 - (3) Examine each concentrated fluid to establish freedom from foreign particles.
5. Shaking of Tests. After the concentrated fluids have been mixed with antigen dilution, shake the tests for a three minute period in the shaking machine.
6. Addition of Salt Solution. After the shaking period, add 0.5 c.c. salt solution to each tube.
7. Reading and Interpretation of Results.
- (a) Read the results as described under the test with serum.
 - (b) Record results in both tubes on the basis of four, three, two and one plus, respectively, depending upon the distinctness of the precipitates.

III. Technic of Presumptive Procedure. The presumptive procedure is made more sensitive than the routine test by the use of a special antigen.

1. Preparation of Special Antigen.

- (a) Extraction of powdered beef heart with ether. Make the four ether extractions as described for standard antigen, except that the ether amounts are 50, 50, 40 and 40 c.c. respectively and the extraction interval between filtrations is five minutes.
- (b) Preparation of Alcoholic Extract.
Same as for standard antigen.
- (c) Cholesterinization.
Same as for standard antigen.

2. Titration of Special Antigen

- (a) The method is in the main like that used for standard antigen. The mixtures of antigen and salt solution used are 1+1, 1+1.1, 1+1.2, 1+1.3 and 1+1.4 respectively.
- (b) After the mixtures have stood for 30 minutes, test the solubility of the precipitate in each by setting up duplicate tubes containing 0.01 c.c. of each mixture and 0.15 c.c. of salt solution.
- (c) Shake the tubes for three minutes.
- (d) Add 0.5 c.c. salt solution to each tube.
- (e) Examine the tubes for precipitates and determine the titer as previously described.

3. Set-up of Tests.

- (a) Preparation of Antigen Dilution. Prepare special antigen dilution for 40 tests at a time and plan to have it ready for pipetting at the time the standard antigen dilution is ready for the routine test.
 - (1) Dilute special antigen with salt solution as described under routine test with serum, using the proportion indicated by the titration of special antigen.
 - (2) Allow the antigen dilution to stand for ten minutes before pipetting.
- (b) Pipetting of Antigen Dilution. Pipette 0.01 c.c. quantities of antigen dilution, one tube for each serum to be tested. Deliver each quantity to the bottom of the tube.
- (c) Pipetting of Serum. Immediately after the antigen dilution has been pipetted, add 0.15 c.c. serum to each tube. (Add each serum to the tube of the presumptive procedure immediately after adding it to the three tubes of the routine test.)

(d) Controls. The controls are the same as for the routine test:

(1) The last antigen dilution quantity +0.15 c.c. salt solution.

(2) Serum controls.

(3) Positive and negative serum controls.

4. Shaking of Tests. After the ingredients are mixed, shake for three minutes in shaking machine.

5. Incubation Period. Place the tests in water bath at 37° C. for fifteen minutes.

6. Reading and Interpretation of Results.

(a) If a test shows a definite precipitate, record the reaction positive.

(b) If there is no precipitate or a doubtful one, record the reaction negative.

IV. Technic of Quantitative Test. In the quantitative procedure a series of serum dilutions is tested with a constant amount of special antigen dilution, to determine the relative quantity of reacting substance in a given serum.

1. Preparation of Serum Dilutions. Prepare dilutions ranging from 1:1 to 1:60 as follows:

1:1 = undiluted serum

1:5 = 0.2 c.c. undiluted serum + 0.8 c.c. salt solution

1:10 = 0.6 c.c. 1:5 dilution + 0.6 c.c. salt solution

1:20 = 0.2 c.c. 1:10 dilution + 0.2 c.c. salt solution

1:30 = 0.2 c.c. 1:10 dilution + 0.4 c.c. salt solution

1:40 = 0.1 c.c. 1:10 dilution + 0.3 c.c. salt solution

1:50 = 0.1 c.c. 1:10 dilution + 0.4 c.c. salt solution

1:60 = 0.1 c.c. 1:10 dilution + 0.5 c.c. salt solution

2. Set-up of Tests.

(a) Set up eight tubes and pipette 0.01 c.c. special antigen dilution into each in the usual manner.

(b) Add the serum dilutions in 0.15 c.c. quantities, in order, to the tubes containing antigen dilution.

(c) Shake the tests for three minutes.

(d) Add 0.5 c.c. salt solution to each tube.

(e) After five minutes, read and record the results in the individual tubes as positive or negative.

3. Interpretation of Results. Express the final result of the quantitative test in terms of reacting units according to the formula $S = 4D$ where S is serum potency in terms of reacting units and D is the highest dilution of serum giving a positive reaction. Table 9 illustrates some reactions with the quantitative procedure.

TABLE 9—SOME TYPICAL REACTIONS WITH THE QUANTITATIVE PROCEDURE

Serum: salt solution.....	1:1	1:5	1:10	1:20	1:30	1:40	1:50	1:60	Four times the maximum dilution of serum giving definite precipitation (4 D)	Serum potency in terms of reacting units (S)
Serum dilution, cc.....	0 15	0 15	0 15	0.15	0.15	0 15	0 15	0.15		
Antigen dilution (special antigen), cc.....	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01		
Serum number										
1	Pos.*	—	—	—	—	—	—	—	4 × 1	4
2	Pos.	Pos.	—	—	—	—	—	—	4 × 5	20
3	Pos.	Pos.	Pos.	—	—	—	—	—	4 × 10	40
4	Pos.	Pos.	Pos.	Pos.	—	—	—	—	4 × 20	80
5	Pos.	Pos.	Pos.	Pos.	Pos.	—	—	—	4 × 30	120
6	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	—	—	4 × 40	160
7	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.	—	4 × 50	200

4. Extremely Potent Serums. If a serum is of unusually high potency, giving definite precipitation in a dilution of 1:60, make still higher dilutions until a negative reaction is obtained.

REFERENCE

1. Serum Diagnosis of Syphilis by Precipitation. Governing Principles, Procedure and Clinical Application of the Kahn Precipitation Test. R. L. Kahn. Williams and Wilkins Co., Baltimore, 1925.

Reporting Wassermann and Kahn Results

1. As soon as the Wassermann findings are recorded, copy a record of these results and of the final results of the Kahn routine test obtained the previous day on the original blanks sent in with the specimens. (Report results with Quantitative and Presumptive procedures of the Kahn test only when requested.)
2. Refer the blanks immediately to a clerk for reporting the same day.

Special Instructions

1. Tests for Repetition.
 - (a) Re-examine with both tests each specimen showing a significant discrepancy in Wassermann and Kahn reactions. Report the results obtained upon retesting.
 - (b) Keep a special record of all repeated examinations, giving the original and final findings and the clinical history, if known.
2. Specimens from Dermatological Clinic, University Hospital, Ann Arbor.
 - (a) Serums.
 - (1) Test each serum giving a strongly positive reaction in the routine test with the Quantitative Kahn procedure.
 - (2) Keep a record of all findings under names of individual patients so that the effect of treatment can be followed in each case.
 - (b) Spinal Fluid Records. Record results of spinal fluid examinations under names of individual patients.

*Pos. =definite precipitation reaction.

- (c) Reports to Dermatological Clinic. Report the findings in all three tubes of the Kahn routine test and also the results obtained with all procedures employed.
- 3. Specimens from Industrial Plants. Keep a record summarizing Wassermann and Kahn results on specimens such as those included in the Motor Wheel survey.
- 4. Special Requests from Physicians.
 - (a) Quantitative Wassermann. Make a series of dilutions 1:2, 1:4, 1:8 and 1:16 and test each dilution, using the regular Wassermann technic. Report the highest dilution giving a four plus reaction.
 - (b) Wassermann on different body fluids. Use the quantities as outlined for spinal fluids. Report results with notation: "These findings have experimental value only."
 - (c) Gonococcus Fixation. Use the Wassermann technic with gonococcus antigen. Report results with the notation: "The complement fixation test for gonorrhea is not at present considered diagnostic."
- 5. Discarding Specimens. Discard serums, clots and other specimens only after results have been reported.
- 6. Unsatisfactory Specimens. Report extremely hemolyzed or contaminated specimens as unsatisfactory for examination.

CHAPTER VIII

OUTLINE OF METHODS OF PROCEDURE IN THE DIVISION OF DIAGNOSTIC MICROBIOLOGY

M. CROOKS, Bacteriologist

The work done in this division covers examinations relative to the diagnosis and control of tuberculosis, gonorrheal infections, throat infections and intestinal infections. Examinations are also made for intestinal parasites, malarial parasites and fungi pathogenic for man. Sanitary bacteriology has been included in this division. The work is limited by the location of Michigan, to communicable diseases peculiar to the temperate climate and to the absence of seaports.

General Considerations

- I. Make a record of procedure on the back of the information blank, signifying the number of examinations by ringed numbers.
 - (1) Stains
 - (2) Culture
 - (3) Animal inoculations
 - (4) Serologic tests
- II. The bacteriologist signing reports is responsible for:
 - (1) All bacteriologic findings reported.
 - (2) Assembly and interpretation of all data accompanying the specimen.
- III. Terminology of reports shall be:
 - (1) Found
 - (2) Not found
 - (3) Specimen unsatisfactory
 - (a) Culture or specimen contaminated
 - (b) Results doubtfulWith unsatisfactory material, make best diagnosis possible; qualify your report, and call for new specimen.
- IV. Clean icebox once weekly.
- V. Disinfect desks after each procedure involving infectious material.
- VI. Dust tops of cupboards, pictures and incubators with antiseptic solution once weekly. This may be done by the janitor under the direction of the bacteriologist.
- VII. Exchange broken supplies and order repairs on broken equipment and fixtures.

- VIII. Senior bacteriologist in charge of the room is responsible for the detailing of the above duties, for the technique of the junior bacteriologists, for the dependability of the stains, for the direction of the janitor in regard to the cleanliness of the room, for posting information on division bulletin board, and for supplying and preparing the direction sheets for the preparation of stains, staining methods and other routine procedure required by the standard methods of the laboratory.
- IX. Change charts in thermograph once weekly, and turn into office, with name of room and date recorded.

Typhoid Fever Diagnosis

I. Agglutination Test. (Widal)¹

1. General.

- (a) If specimen permits, a macroscopic agglutination test shall be made.
- (b) Subculture daily in plain extract broth and on plain agar slant, Dorset's strain of *B. typhosus*. (Standardized suspension of killed bacteria may be used.)²
- (c) Equal parts of physiologic salt solution and culture of typhoid bacilli shall be used as a positive control, equal parts of immune typhoid serum and culture of typhoid bacilli shall be used as a positive control, at the time the tests are made.
- (d) Stock culture shall be plated for purity and titer be determined and reported at least every month.
- (e) Five hour culture of motile organisms shall be used for agglutinations.
- (f) At least two dilutions of serums shall be used, 1-20 and 1-40.

2. Dried Blood Specimens. (Microscopic)

(a) Dilutions.

- (1) To one drop of dried blood add one drop of physiologic salt solution with a capillary pipette.
- (2) To one loopful of this preparation add 9 loopsful salt solution for a 1-10 dilution, taking care not to touch loop to serum while adding salt solution.
- (3) Stir thoroughly with loop.
- (4) Dilute one loopful 1-10 dilution with one loopful salt solution making a 1-20.

For test

- (5) To one loopful of serum (1-20 dilution) add one loopful of typhoid culture making a final dilution of 1-40.

II. Routine Procedure for Isolation of Typhoid, Paratyphoid, Dysentery and Morgan's Bacilli.

1. Preparations of specimens for plating.³

- (a) Feces.
 - (1) Emulsify small portion of stool in peptone solution, making a dilution of approximately 1-15; streak plates at the end of 15 minutes.
 - (2) If feces is diarrhea-like, streak from undiluted and from diluted specimens.
 - (3) Plates are not satisfactory unless they show isolated colonies for fishing.
- (b) Blood culture. (Bile medium recommended. Bile+10%, glycerin+2% peptone.)
 - (1) Make hanging drop from broth culture after 24 hours' incubation, if motile rods are present,
 - (2) Make smear, stain by Gram's method. If Gram negative rods are present,
 - (3) Run agglutination tests, and inoculate XXX sugar medium
 - (4) Plate by adding 0.1 c.c. of culture to Endo plate and blood plate with sterile pipette and streak with sterile loop.
 - (5) Report the above procedure at the end of 72 hour incubation, if the previous results are negative.
- (c) Urine.
 - (1) Make hanging drop. If motile organisms are present run macroscopic agglutination tests.
 - (2) Plant in broth and incubate for two hours and plate, or sediment by centrifugalization and plate from sediment.

III. Plating.

- 1. For each specimen at least three plates shall be used, and two medium consisting of one Endo plate and two Krumwiede's brilliant green. Standardize brilliant green monthly and pour plates daily.
- 2. Incubate plates 18-24 hours at 37 degrees Centigrade.
- 3. As controls, plate positive feces or feces plus culture of *B. typhosus* on both medium.

IV. Fishing of Colonies.

- 1. After incubation examine plates under hand lens.
 - (a) Typical or suggestive colonies are fished with a platinum needle and transferred to XXX sugar medium, inoculated by a deep puncture into the butt and along the surface of the slant.
 - (b) Incubate tubes 18-24 hours.

V. Motility Tests.

- 1. Read reaction of XXX sugars. (Control purity of culture by stained smears.)
- 2. If any reactions are typical of typhoid, paratyphoid, dysentery, or Morgan's bacilli
 - (a) Broth is added to the slants and

- (b) Hanging drop made from the suspension of organisms.
- (c) If organisms are not motile, incubate four hours and test for motility again.

VI. Agglutination Tests.

1. If organisms are motile, agglutination tests are made with typhoid immune serum, and with paratyphoid immune serums if sugar reactions are typical of the paratyphoid group.
- *2. If organisms are not motile and XXX sugar reactions are typical, agglutination tests are made with dysentery polyvalent and Shiga immune serums; thereby, differentiating between the true Shiga strain and the para dysentery strain.
3. Incubate test two hours and observe every half hour, then place in icebox overnight.
4. Results of agglutination tests.
 - (a) Preliminary report may be made upon three dilutions and negative and positive controls, titer to be determined later.
 - (b) If an organism with characteristic morphology, staining properties and sugar reactions of typhoid bacilli, paratyphoid bacilli or dysentery bacilli fails to agglutinate with its specific serum, check sugar reactions, test for indol production and reaction in litmus milk, transfer it in pure culture to plain agar, and after making several subcultures try agglutination tests again. If the culture has the cultural characteristics of *B. Morgani*, test agglutination reaction with immune *B. Morgani* serum.
5. Reports of examinations shall be:
 - (a) *B. typhosus*, *B. paratyphosus A*, *B. paratyphosus B*, *B. dysenteriae*, or *B. paradysenteriae* found.
 - (b) Organisms of the typhoid-dysentery group not found.
 - (c) *Morgan's bacillus* found. This positive finding may be based upon cultural characteristics, without serologic confirmation. Request sample of patient's serum for agglutination test for confirmation.

NOTE: Save feces overnight for further work if indicated. Save for study, all non-agglutinating organisms having the cultural characteristics of the typhoid-dysentery group, or of *B. Morgani*. Put all positive cultures in stock. Give the culture with stock-culture card completely filled out to curator.

*Due to the cross agglutination of the various strains of paradysentery, no attempt is made to differentiate before reporting routinely. The culture should be classified by sugar reactions (Dextrose, Mannite, Maltose, Suchrose) and by absorption tests and put in stock.

REFERENCES

1. Pathogenic Micro Organisms. Park and Williams. () See chapter on Agglutination. Lea and Febiger, 1924.
2. Ibid. Page 202.
3. Journal Infectious Diseases. 23:275, 1918. Krumwiede et al.

Routine Examinations of Throat Swabs

Comparative studies of diphtheria cultures on Loeffler's medium with the original swabs transported by mail showed the diagnostic value of culture and swab to be equal. The swabs offer, however, many advantages; notably, the direct microscopic examination, the diagnosis of Vincent's Angina, and the blood plate. An analysis of our records on diagnosis in throat infections demonstrates the value of the direct microscopic examination, and the blood plate in the laboratory diagnosis of throat infections.

I. Swabs for Diagnosis.

Swabs from cases giving clinical history of throat infection shall be classed as diagnostic (diphtheria, septic sore throat, tonsillitis, scarlet fever, quinsy, Vincent's Angina, etc.)

Diagnostic swabs are examined for *B. diphtheriae*, streptococci, and organisms of Vincent.

Preparation of Media:

1. Blood plates (5% sheep's blood).

Agar Beef Extract+NaCl (pH 7.0) 100 c.c.

Sterile sheep's blood 5 c.c.

2. Loeffler's Serum Medium.

Filtered Beef Serum 3 parts

Broth Beef Ext. +1% Dextrose 1 part

Adjusted to pH 6.8—7.2. Slanted in Arnold, maximum temp. 75°. Sterilized in autoclave by running pressure slowly up to 10 lb., holding for 45 minutes, then to 15 lb. for 15 minutes.

Procedure:

1. Inoculate, first, blood plate; second, inoculate Loeffler's serum; third, make smear on slide.
2. Examination of direct smears:
 - (a) Stain with Loeffler's methylene blue.
To stain: Dry and fix smear on slide. Flood slide with stain for 2 minutes, wash in water, dry in air.
 - (b) Report only positives on direct smears unless a report is requested, then make a provisional negative report.
 - (c) Report Organisms of Vincent when fusiform bacilli are found in the presence of spirocheta.

II. Swabs for Release.

1. Throat swabs for release include release from diphtheria quarantine, release from isolation for septic sore throat and isolation for carrier.
 - (a) Releases from diphtheria quarantine and isolation as diphtheria carrier are inoculated on Loeffler's serum media.

- (b) Releases from isolation for septic sore throat and from isolation as carriers of streptococci are inoculated on both Loeffler's medium and blood plates.

III. Swabs for the Identification of Carriers.

Swabs from the following sources shall be considered as "carriers."

- (a) Persons not ill but suspected of carrying virulent diphtheria bacilli.
- (b) Contacts with cases of diphtheria, septic sore throat and scarlet fever.
- (c) Routine throat swabs from state institutions unless a clinical history is given.
- (d) Routine throat swabs from nose and throat specialists.

IV. General Ruling.

1. At 8:00 a.m. daily or after 10-18 hours incubation, spread smears, stain with Loeffler's alkaline methylene blue and examine.
2. Any cultures which show solid forms (A2 and C2) or overgrowth of other organisms should be smeared again and stained by a differential stain to demonstrate granules. Cultures showing granules and doubtful morphology should be stained by Gram's method.
3. Read blood plates. Report hemolytic and green producing streptococci on diagnostic swabs. (See classification of streptococci, p. 91.)
 - (a) Report hemolytic streptococci on swabs from releases and contacts in scarlet fever cases and septic sore throat. (Carriers.)

V. Guinea Pig Inoculations for Virulence of B. Diphtheriae.

1. Run virulence tests on
 - (a) All quarantine releases of eight weeks' duration, or on request.
 - (b) On cases not giving a history of infection (carriers).
 - (c) On atypical forms when clinical diagnosis is doubtful.
 - (d) On cases from state institutions.
2. Guinea pigs weighing 250-300 grams shall be used for virulence tests.
3. 1.0-1.5 c.c. of broth suspensions of a 24 hour culture of Loeffler's may be injected subcutaneously into two guinea pigs one of which has been previously inoculated with a protective dose of antitoxin.
4. The routine Loeffler's may be used if the slant shows a heavy growth of B. diphtheriae.
5. Inoculate a Loeffler's plate at the time of animal inoculation and

use as control of inoculum. Isolate B. diphtheriae in pure culture to be used as check when routine virulence test is unsatisfactory. (See 6 (b) (4)).

6. Diagnosis.

(a) All pigs inoculated shall be autopsied at death or at the end of 72 hours.

(b) Interpretations of autopsies.

- (1) Death from toxemia, showing edema, engorged suprarenals and necrosis at site of inoculation shall be considered *positive*.
- (2) The failure to gain weight and the signs of toxemia to any degree when pig is killed and brought to autopsy shall be considered *positive*.
- (3) Pigs dying or sick from other causes, or normal at autopsy shall be considered *negative*, except when pig dies in less than 24 hours, or control culture shows no diphtheria-like organisms.
- (4) Pigs dying in less than 24 hours without typical lesions of diphtheria toxemia or when control culture shows no organisms shall be considered *unsatisfactory*.

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Bacteriological Examination for B. Tuberculosis

I. Sputum.

1. Report physical findings. (See Chemical Diagnosis by Wood. p. 381.)
- *2. Sterilize all specimens except those, the character and history of which indicate making culture or animal inoculation. Add 10 c.c. of one per cent sodium carbonate in one per cent phenol solution to five c.c. sputum before sterilization if specimen is tenacious.
3. Remove supernatant fluid from sterilized specimens and make smears from the sediment using one slide for one smear, and stain by Ziehl-Neelson method.
4. Save slides and specimens until reports are made.
5. Report the absence or presence of cells and note predominating type.

Caution: Four or five acid-fast bacilli do not constitute a positive finding and should be confirmed. Swabs are not satisfactory for diagnosis.

6. Animal inoculation. Inoculate guinea pigs when requested by physician. Treat specimen with antiformin.

II. Urine. Urine is examined for tubercle bacilli, when tuberculosis is suspected, or clinical diagnosis is doubtful.

1. Report physical findings.
2. Smear. Centrifuge and make smears from sediment. Use serum for fixing smears.
 - (a) Stain smear by Gram's method.
 - (b) Stain smear by Ziehl-Neelson.
 - (c) Wet preparation.

Report "acid-fast bacilli 'found' or 'not found'". Smear findings are not conclusive and animal inoculation should be made.

3. Save all slides positive on direct examination. File in Room 710.
4. Culture. Culture sediment as a control of material for animal inoculation, and for diagnosis of infections of the urinary tract other than tuberculosis.
 - (a) Streak blood plate.
 - (b) Streak Endo plate.
5. Animal inoculation. Make animal inoculation to confirm findings of acid-fast bacilli, or as further aid to diagnosis when acid-fast organisms are not found. Treat sediment with antiformin unless culture of sediment shows no growth.

*Jr. Lab. and Clin. Med., 6:41, Oct., 1920.

III. Transudates and Exudates. (Various body fluids, ex. spinal, pleural, etc.)

All specimens are examined for tubercle bacilli when clinical diagnosis is doubtful or tuberculosis is suspected.

1. Report physical findings. (Diagnostic Methods by Webster. (p. 736.)

2. Procedure.

(a) Clear fluid or fluid with flocculent sediment:

(1) Centrifuge.

(2) Make three smears.

a. Stain by Wright's method for differential cell count.

b. Stain by Gram's method.

c. Stain by Ziehl-Neelson.

(3) Culture. As in Urine.

(4) Animal inoculation. As in Urine.

(b) Fluid with coagulum: (Characteristic of tuberculosis).

(1) Digest coagulum.

(2) Centrifuge, wash and make smear from sediment.

(3) Stain smear by Ziehl-Neelson method.

(4) Inoculate guinea pig as a further aid in diagnosis.

(c) Pus. Pus is associated with so many pathological conditions other than tuberculosis that every specimen should receive general bacteriological examination, including an examination for ray fungus, before proceeding with technique for tubercle bacilli.

(1) Treat with antiformin.

(2) Centrifuge and wash sediment—at least 3 washings.

(3) Make smear and stain by Ziehl-Neelson.

(4) Save all positive smears. File in Room 710.

(5) Animal inoculation. Inoculate guinea pig to confirm findings of acid-fast bacilli, or as a further aid in diagnosis.

IV. Animal Inoculation.

1. General.

(a) All cages containing inoculated animals shall be labelled with tag giving date of inoculation, ear tag number, name of doctor, kind of specimen and name of worker. The outline on back of tag shall show characteristic color markings of the animal. The tag is to be securely fastened upon the cage.

(b) Each pig shall be identified by ear tag.

- (c) Record of animal inoculation shall be kept on cards. This card carries doctor's name and address, patient's name, kind of specimen, date received, preliminary findings, date of inoculation, amount of inoculum, method of inoculation, pig number, and name of worker. This animal inoculation card shall be kept in bacteriologist's file until animal is autopsied, at which time it will be used for autopsy report. The card will then be turned into the office for reporting and filing.
 - (d) An animal inoculation card shall be filed for each animal used for experiment or research, as well as for diagnostic purposes. In the case of experimental work, the character and result of the experiment shall be recorded on card.
2. Procedure.
- (a) Inoculate guinea pig weighing 350-450 grams with 1-2 c.c. saline emulsion of sediment subcutaneously in groin after bruising inguinal gland to render more susceptible to infection.
3. Diagnosis.
- (a) Weigh pig at time of inoculation. All inoculated pigs shall be examined and weighed weekly, and observations recorded on back of animal inoculation card.
 - (b) Kill pig and perform autopsy when there is loss of weight and enlargement of glands.
 - (c) If pig is normal at the end of 4 weeks, report same to doctor and observe pig two weeks longer.
 - (d) At the end of 6 weeks, kill pig, perform autopsy and report unless condition indicates further observation.
 - (e) Make positive diagnosis on the presence of tubercles and demonstration of tubercle bacilli in spleen and glands.
4. Examination shall be reported:
- (a) "Date of inoculation, number of pig, guinea pig died, or was killed, B. tuberculosis 'found' or 'not found' at autopsy, date."
 - (b) "Date of inoculation, number of pig, guinea pig showed no symptoms to date, date of reporting. We would like to observe this animal for two weeks longer."
 - (c) If pig dies before end of incubation period, perform autopsy, and report "Guinea pig (number of pig), inoculated (date), died (date) before expiration of incubation period. Cause of death."

The following texts are supplied by the department: Pathogenic Micro-Organisms by Park and Williams; Diagnostic Methods by Webster; and Practical Bacteriology by Stitt.

Diagnosis of Gonorrheal Infection

I. Smears for Gonococci.

1. Stain diagnostic smears by Gram's method, number slide with diamond pencil to correspond with number on blank, report intracellular Gram negative diplococci as gonococci, report extracellular Gram negative as such.

NOTE: Plus and minus may be substituted for the terms "found" and "not found."

2. Save all slides.

NOTE: The diagnosis shall be made only when organisms are intracellular and morphological characteristics, grouping, and staining reactions are typical. The reliability of the staining reagents shall be, in every case, controlled by testing them with smears of known Gram positive and Gram negative bacteria. It is necessary to bear in mind the fact that the discharges from mucous membranes, commonly the site of infections with the gonococcus, not infrequently contain Gram negative cocci which are not gonococci.

3. Report pus cells as "many found" or "found" or "few found."

NOTE: Dry swabs and vaginal discharges for smears are not satisfactory.

4. All smears for gonococci shall be examined by two microscopists; all differences referred to the Senior Bacteriologist.

II. Cultures.

1. When possible to secure material for culture for gonococci, confirm smear by culture.
2. When organisms found in smears suggest infections other than gonorrhea request material for culture.

III. Smears for Gonorrheal Ophthalmia. See I, 1.

1. Stain by Gram's method.
2. Report predominating organisms, ask for cultural material when organisms on smear suggest infections other than gonococci.

Reference: Williams and Rosenberg: Arch. of Ophthal. 1916, XIV, 109.

IV. Urine for Gonorrheal Infection.

1. Make smears from centrifuged sediment.
2. Stain by Gram's method.
3. Report gonococci as indicated under smear reports.

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Bacteriological Examination of Water

I. For test, use:

1. Two agar plates.
 - (a) Raw water and sewages, plate in duplicate.
2. Five Dunham's fermentation tubes ($6 \times \frac{3}{4}$) containing double strength lactose broth.
3. One Dunham's fermentation tube ($5 \times \frac{5}{8}$) containing single strength lactose broth.
 - (a) Raw waters 5-1 c.c., 5-1/10 c.c.

II. Procedure.

1. General: Flame top of bottle, shake 25 times vigorously.
2. Presumptive test.
 - (a) Inoculation.
 - (1) Place 1 c.c. of sample in each of two Petri dishes.
 - (2) Place 1 c.c. of sample in tube containing single strength broth.
 - (3) Place 10 c.c. of sample in each of 5 tubes containing double strength broth.
 - (b) Incubation.
 - (1) Incubate at 37 C. for 48 hours.
 - (c) Interpretation of results.
 - (1) Safe—all tubes are negative.
 - (2) Unsafe—all tubes are positive.
 - (3) Suspicious, which may be
 - a. Reported, in case of low count and fermentation in not more than one tube.
 - b. Confirmed, in case of high count or slight fermentations in more than two tubes.
3. Confirmatory test.
 - (a) Partial.
 - (1) Endo's medium or Eosin Methylene blue agar.
 - a. Transfer 3 loopsful from tube showing gas from smallest amount of water tested, or tube showing gas with least turbidity and smallest pellicle, to a tube of sterile water (3 c.c.) From this, streak plates.
 - b. Incubate at 37 C. for 24 hours.
 - c. If typical metallic red colonies appear, record as positive; if no typical colonies, record as —— and confirm further.
 - (b) Completed.
 - (1) Refermentation.
 - a. Fish at least 2 typical colonies and transfer to a

Dunham's fermentation tube containing single strength lactose broth.

- b. Incubate at 37 C. until gas formation is noted; the incubation not to exceed 48 hours.
- c. Presence of gas at end of incubation demonstrates the presence of a member of B. coli group.

REFERENCE

Standard Methods of Water Analysis. A. P. H. A.

Bacteriological Examination of Milk

I. Apparatus, for each sample.

1. Two tubes containing 9 c.c. of sterile water.
2. Two Petri dishes.
3. One flask containing 200 c.c. sterilized water.

II. Routine Procedure.

1. Flame neck of milk bottle and shake with rotary motion fifty times, flame again.
2. Dilutions.
 - (a) Pipette 1 c.c. milk into dilution flask. Shake 25 times.
 - (b) Pipette 1 c.c. of sample from dilution flask into
 - (1) Petri dish (1:200).
 - (2) Dilution tube. Shake 25 times.
 - (c) Pipette 1 c.c. from dilution tube (b(1)) into
 - (1) Petri dish (1:2000).
 - (2) Second dilution tube. Shake.
 - (d) Pipette 1 c.c. from dilution tube (c(1)) into
 - (1) Petri dish (1:20,000).

3. Plating.

- (a) Cool agar to a temperature of between 40° and 45° C. before using. Pour about 10 c.c. of the melted agar to the plate and mix agar and milk by a gentle rotary motion.

4. Incubation.

- (a) Incubate 36 to 48 hours at 37.5° C.

5. Report.

- (a) Render report in round numbers only.
- (b) Results are expressed as "colonies per c.c." or "official plate count" so much per c.c.

III. Direct Enumeration of Bacteria in Milk.—Prescott and Breed.

1. Procedure.

- (a) Spread .01 c.c. milk from well-shaken sample on 1 sq. cm. of slide—duplicate on same slide.
- (b) Dry on level surface.

- (c) Remove fat with xylol—wipe off surplus with filter paper.
- (d) Dry and fix in 95 per cent alcohol.
- (e) Stain two or three minutes in Loeffler's Methylene blue.
- (f) Decolorize to light blue in 95% alcohol.
- (g) Count under oil immersion lens.

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4. Sediment Test. Ref.: Standard Methods of Milk Analysis. Am. Public Health Assoc. pp. 20-21, 1923. Heineman, Milk. pp. 200-207.
5. Detection of Specific Pathogens in Milk. Standard Methods of Milk Analysis. Am. Public Health Assoc. pp. 21-22, 1923.

General Bacteriological Examinations

- I. Blood for diagnosis in septicemia and pneumonia. Keidel tube containing dextrose broth is recommended for transporting blood for culture through the mails.
 1. Incubate blood in dextrose broth until growth appears or for 72 hours.
 - (a) A broth culture should be incubated not longer than 12 hours before observation, for pneumococci autolyzes upon prolonged incubation.
 2. When growth appears, subculture on blood plate and make smears.
 3. If no growth appears, make sub-culture on blood plate at end of 48 hour incubation by inoculating plate with 0.3 c.c. broth culture after shaking.
 4. Diagnosis:
 - (a) Follow necessary procedure for the identification of organisms recovered.

(b) The following are the most important organisms recovered in culture:

- (1) Pneumococci. If culture shows diffuse growth and smear shows Gram positive diplococci which are soluble in bile, follow procedure for typing pneumococci in blood. Ref. Rockefeller Monograph No. 7.
- (2) Streptococci. If culture shows granular growth and Gram positive cocci or diplococci in chains, insoluble in bile, classify streptococci by key to identification of streptococci in blood plates. Ref. Rockefeller Monograph No. 9.
- (3) Typhoid bacilli. See Typhoid Fever Diagnosis.
- (4) Staphylococci. Identified by smears and growth on blood plate. Due to the wide distribution of this organism, staphylococci is of little etiological significance unless confirmed.
- (c) Contaminated cultures. If cultures are so overgrown with hay bacilli that the diagnostic findings are obscured, report "Culture contaminated."

II. Urine for diagnosis of pyelitis, cystitis (not including tuberculosis).

1. Note physical findings.
2. Centrifuge.
3. Make wet preparation of sediment for studying cells.
4. Make smear for Gram's stain.
5. Culture. (Only catheterized specimens are of diagnostic value.)
 - (a) Inoculate dextrose bouillon tube, blood plate and Endo's plate.
 - (b) Identify organisms recovered.

III. Sputum.

1. Bronchitis, influenza and lung abscess.
 - (a) Report physical findings.
 - (b) Examine smear stained by Gram's method.
 - (c) Inoculate blood plate.
 - (d) Identify predominating organism.
2. Pneumonia.
 - (a) Type sputum. (See Pneumonia Routine.)
 - (1) Avery's method.
 - (2) Mouse method.
3. Asthma.
 - (a) Examine wet preparation for spirals and crystals.
 - (b) Differential stain for eosinophiles.
 - (c) Culture blood plate for predominating organism.

IV. Pus. Specimens of pus may be received on swabs or in varying amounts. (Specimens from nasal discharge and ears are referred to division of throat infections.)

1. Make wet preparation for direct examination for actinomyces if granules are observed.
2. Examine smear stained by Gram's method, report predominating organisms and the type of cells found.
3. Culture on blood plate and identify predominating organism.

V. Diagnosis in Meningitis.

1. Tuberculosis meningitis. (See Bacteriological Examination in Tuberculosis.)
2. Epidemic cerebro-spinal meningitis. (Keep specimens warm.)
 - (a) Note character of specimen. The fluid may be flocculent or contain considerable pus.
 - (b) Cell count. A cell count is not satisfactory unless specimen is fresh and free from blood.
 - (c) Examine smear stained by Gram's method for Gram negative intracellular diplococci. Make differential cell count.
 - (d) Culture.
 - (1) 0.5 c.c. dextrose broth culture.
 - (2) Inoculate warm freshly poured dextrose blood agar plates with 0.5 c.c. fluid.
 - (3) Place remainder of specimen in incubator for 12-24 hours.
 - (4) Incubate cultures under modified oxygen tension.
3. Spinal fluid for etiological organism.
 - (a) Centrifuge and make smears from sediment.
 - (b) Examine smears stained by Gram's method.
 - (c) Make differential count.
 - (d) Make dextrose-broth culture and inoculate blood plate.

NOTE: Nasopharyngeal swabs from contacts should be examined for identification of carriers, and from patients as an aid to diagnosis.

VI. Diagnosis in Empyema. (Compare Tuberculosis Diagnosis.) Follow "Pneumonia Routine."

1. Sediment specimen by centrifugation unless specimen is purulent.
2. Examine smear stained by Gram's method.
3. If Gram positive diplococci are present, proceed with pneumococcus typing.
4. Culture for predominating organism.
 - (a) If no bacteria are present in smear, inoculate dextrose broth plus 5 per cent sterile blood.
 - (b) If bacteria are present in smear, streak blood plate.
 - (c) Identify organism.

Miscellaneous

There are an appreciable number of specimens received from various sources sent in for bacteriological examination. Each specimen presents an individual problem, and the examinations made depend upon the character of the specimens and the reason for the examination. These specimens in the past have been dressings and sutures for sterility tests, brushes for anthrax, tonsils for infectious organisms, et cetera.

Caution: Save slides, specimens, and cultures until final diagnosis is made and *reported*.

NOTE: Save slides showing *Treponema Pallida*. Label and file in room 710. Put in stock, cultures of diphtheria-like organisms from sources other than throats; streptococcus viridans from blood cultures and spinal fluids; pneumococcus, streptococcus hemolyticus, meningococcus, and gonococcus from all sources.

Stock Cultures

Stock cultures shall consist of the following organisms and be numbered as indicated in Key to Stock Culture Numbers.

Key to Stock Culture Numbers

Stock Numbers	Group.	Stock Numbers	Group.
201-300.....	Streptococcus.....	1101-1200	Crooks
301-400.....	B. Diphtheria.....	1201-1300	Kendrick
401-500.....	Miscellaneous.....	1301-1400	Meningococcus
501-600.....	Typhoid-Intermediate.....	1401-1500	Gonococcus
601-700.....	B. Coli.....	1501-1600	Pneumococcus
701-800.....	B. Typhosus.....	1601-1700	B. Influenzae
801-900.....	B. Paratyphosus A.....	1701-1800	B. Tuberculosis
901-1000.....	B. Paratyphosus B.....	1801-1900	Henry
1001-1100.....	B. Dysenteriae.....	2001-2100	Marshall
		2101-2200	Streptococcus Viridans

Records:

A card giving complete information as to source of culture, shall accompany culture when handed to curator for stock. Cards consist of two kinds, "Stock Cultures" and "Virulence." These cards shall be filed with all records pertaining to stock cultures by curator in efficiency desk, Room 710.

A memorandum shall be sent to Bacteriologist of any cultures lost from stock.

A record of transfer and condition of cultures shall be kept in ink in a book provided for that purpose.

Note on cards to whom cultures are sent and give date.

Cultures:

Cultures shall not be removed from stock except by curator, the curator to be responsible to Bacteriologist or Assistant Bacteriologist.

Cultures shall be kept in ice box, except as otherwise specified, in Room 705, each tube numbered with wax pencil and sealed with paraffin when indicated, in copper racks labelled with tags.

Inspect cultures weekly for moulds.

Media:

Only fresh media carefully checked for contamination shall be used for preserving stock cultures.

1. *Streptococcus Hemolyticus*:

Media: Nutrient semi-solid. Transfer every 4 weeks.

2. *B. Diphtheriae*:

Media: Loeffler's serum. Transfer every 3 weeks.

3. Typhoid-Colon Group:

Media: Semi-solid, or 1½% beef extract agar slants. Transfer every two months.

4. *Gonococci*, *Meningococcus*:

Media: Coagulated blood. Transfer every 4 days.

5. *B. Influenza*:

Media: Chocolate medium (Park and Williams). Transfer every 7 days.

6. *B. Tuberculosis*:

Media: Dorset egg. Transfer: Start new cultures every 3rd week.

7. *M. Catarrhalis* (Miscellaneous):

Media: Coagulated blood. Transfer every two weeks.

8. *Streptococcus Viridans* and *Pneumococcus*:

Media: Coagulated blood slants. Transfer every week.

Pneumonia Routine

Routine of procedure for determining etiological organism in pneumonia and the typing of pneumococci. The organisms recognized as the most important, as the etiological agent in pneumonia are: *Pneumococci*, *streptococcus viridans*, hemolytic streptococci, Friedlander's bacilli and influenza bacilli.

I. General.

1. Make two smears, one to be stained by Gram's method and one by Hiss' capsule stain.
2. Inoculate blood plate.
3. Plant 0.5 c.c. specimen in Avery tube for Avery's method of typing. Incubate 5-12 hours at 37° C.
4. Inoculate 0.5 c.c. into peritoneal cavity of mouse.
5. At the end of 5 hours or when growth appears, make smear and centrifuge Avery tube at low speed for 2 or 3 minutes to throw down red cells and pus. Draw off supernatant fluid for agglutination, and precipitation tests. Make cultures on one-half blood plate from supernatant fluid and on the other half from sediment. If bile soluble Gram positive diplococci are present, run agglutination tests. If smear shows mixed culture run precipitin tests.

6. If Avery culture is negative identify predominating organism on blood plate.

II. Sputum.

1. Carefully wash sputum through three or four washings of saline solution and with a few drops of saline solution make a thick emulsion of same. For Procedure, see General, 1-6 inc.

III. Typing.

1. Precipitin test.

- (a) Take 2.5 c.c. of above culture and 1 c.c. of oxbile, mix in sterile tube and place in water bath at 37° C. for twenty minutes. In the meanwhile set up four tubes, as follows:

Tube 1—.3 c.c. of Undiluted Serum, Type 1.

Tube 2—.3 c.c. of Undiluted Serum, Type 2.

Tube 3—.3 c.c. of Serum, Type 2, Diluted 1:20.

Tube 4—.3 c.c. of Serum, Type 3, Undiluted.

- (b) Add to each tube .3 c.c. of bile and culture mixture and place in water bath at 37° C. At the end of 20 minutes to half hour precipitin reaction should have taken place. Smears should be made from Avery culture and also from bile mixture and examined for organisms.

2. Agglutination tests:

- (a) Set up four tubes, as follows:

Tube 1—.3 c.c. of Serum, Type 1, Diluted 1:20.

Tube 2—.3 c.c. of Serum, Type 2, Undiluted.

Tube 3—.3 c.c. of Serum, Type 2, Diluted 1:20.

Tube 4—.3 c.c. of Serum, Type 3, diluted 1:3.

Fifth tube place .2 c.c. of oxbile.

- (b) To each tube add .3 c.c. of Avery culture and set in water bath for from one to two hours, at end of which time examine for agglutination and bile solubility. Vary dilutions of sera according to titer as indicated by manufacturer on label.

3. Mouse method:

- (a) At the end of eight hours, if mouse is not dead, kill the animal and proceed. Open the peritoneal cavity of mouse under sterile conditions and make culture on blood agar slant, one-half of blood agar plate and make smears on two slides, examining one by Gram's method, the other by capsule stain. Wash out cavity with sterile saline solution and collect about 5 c.c. of the washings.
- (b) Centrifuge at low speed for two or three minutes, just enough to throw down pus draw off supernatant

liquid and divide into two portions. With one portion set up agglutination test as above and proceed with other portions, as under precipitin test, incubating as before. Carefully expose heart of mouse and sear, snip off the tip and at once make culture on blood agar slant, remaining half of blood agar plate and inoculate a tube of sterile broth with blood. Also make two smears as above.

3A. Modification of Mouse Method (Major Butler).

- (a) Centrifuge peritoneal washings at low speed for two minutes, draw off supernatant fluid into sterile tube and centrifuge at high speed for five minutes and set up precipitin test with supernatant liquid. Re-emulsify sediment with sterile saline solution and after centrifuging at low speed for about one or two minutes, to throw down pus and cells, set up agglutination tests.

IV. Blood Culture. See General, 5 and 6.

V. Spinal Fluid and Empyema Fluids. See General, 1-6 inclusive.

VI. Media. See Rockefeller Monograph No. 7.

Concentration Methods

I. Sedimentation of sputum by sterilization.

1. Number specimen jars with diamond pencil, and information blanks with wax pencil to correspond.
2. Add saline to specimen when small.
3. Arrange specimens in boiler so that there is no danger of one specimen boiling over into another.
4. Cook for one hour in utensil sterilizer.

II. Antiformin method.

1. Antiformin.

(a) Liquor Sodae Chlorinatae

Sodium carbonate.....	300 gm.
Chlorinated lime.....	200 gm.
Distilled water.....	2000 ml.

Dissolve the sodium carbonate in 500 mls of the distilled water. Thoroughly triturate the chlorinated lime in remainder of the water. Mix the two and filter again.

- (b) Caustic soda solution 15%. Mix equal parts of (a) and (b).

2. Procedure.

- (a) Sputum. One part antiformin to 5 parts sputum. Shake thoroughly and allow to stand about 20 hours. Centrifuge and wash sediment twice.

- (b) Urine, pus and body fluids. Treat sediment with 20% or 25% antiformin and let stand 3-18 hours. Wash at least twice.

Anaerobic Methods

I. Partial oxygen tension (Meningococci, streptococci, gonococci).

1. Deep agar tubes or broth tubes.
 - (a) 1% glucose agar in 5x5/8 tube.
 - (b) Boil just before using to expel oxygen.
 - (c) Cool quickly to 40-45° C. by placing tube in cold water.
 - (d) Inoculate and solidify at once in ice water.
 - (e) Add layer of sterile paraffin or mineral oil.
2. To produce atmosphere containing 10% CO₂. Kohman, Jr. Bact. 1:67, 1916.
 - (a) Use desiccator for plates.
 - (b) Place 5 gms. Na₂CO₃ in jar.
 - (c) Pour over it a solution of 5 mls H₂SO₄ and water 35 mls.
 - (d) When reaction begins to subside place air-tight cover on jar.

II. Pyrogallic methods.

1. Buchner's.
 - (a) Use 1 gm. pyrogallic and caustic soda for every 100 c.c. of space in jar or tube to absorb the oxygen.
 - (b) Desiccator may be used for Petri dishes or large number of culture tubes, or 8x1 test tubes for cultures in 5x5/8 and in 3½x½ tubes.
2. Wright's.
 - (a) Use deep agar tubes as in I. 1.
 - (b) Burn cotton plug and push it down leaving a space of about one inch from top of tube.
 - (c) Fill space with pyrogallic acid, add 2 mls 10% caustic soda.
 - (d) Quickly insert rubber cork.

Key to the Identification of Streptococci on Blood Plates

Gram positive cocci arranged in pairs or chains growing well only on enriched media.

- | | |
|---|-------------------------------------|
| A. Methemoglobin produced. | <i>Green-producing streptococci</i> |
| B. Methemoglobin produced with hemolysis. | Type I. |
| (Alpha Type, Brown's Classification) ¹ | |
| BB. Methemoglobin produced without hemolysis. | |
| C. Bile Soluble. | <i>Pneumococci</i> ² |
| D. Agglutinated in immune pneumococcic sera. | Types I, II & III. |

- DD. Not agglutinated in immune pneumococcic sera. Type IV.
 CC. Not soluble in bile. *Streptococcus Viridaus*
 AA. Hemolysis produced. *Streptococcus hemolyticus*
 B. Colonies surrounded by a narrow zone of hemolysis. Type II.
 C. Minute punctate colony. Type IIa.
 CC. Large colony, 1-2 mm. in diameter. Type IIc.
 BB. Colonies surrounded by a wide zone of hemolysis. Type III.
 (Beta hemolytic streptococci, Brown's Classification)
 C. Minute punctate colony. *Type IIIa.
 CC. Large colony. Type IIIc.
1. Monograph No. 9, Rockefeller Institute for Medical Research.
 2. Monograph No. 7, Rockefeller Institute for Medical Research.
 3. The Blood Plate as an Aid to Laboratory Diagnosis. C. C. Young and Minna Crooks, Michigan Department of Health. Jr. Lab. and Clin. Med., 9:409. (March, 1924.)

Standardization of Brilliant Green Media

- I. Each batch of agar should consist of sufficient amount to meet a 3 to 4 weeks demand as determined by bacteriologist in charge of media. Fill into 10 French Squares, 100 mils each, at time of preparation. Store remainder into French Squares containing measured amounts.
- II. Stock solution of brilliant green should be prepared fresh at each standardization.
- III. Procedure:
 1. Medium: Melt 3 French Squares of 100 mils each. Add andrade indicator, amount as determined by titration of product in use. Add sterile solution (Lactose 20%, Glucose 2%) 5 mils. To Flask No. 1, add .1 mil of a .1% brilliant green solution = 1 to 1,000,000.
 To Flask No. 2, add .3 mil of a .1% brilliant green solution = 1 to 330,000.
 To Flask No. 3, add .5 mil of a .1% brilliant green solution = 1 to 200,000.
 Pour 5 or 6 plates from each bottle in routine manner.
 2. Inoculation. The best material for standardization is positive stools from typhoid carriers or cases, and should consist of one paratyphoid "B" and two typhoids. Stools should be freshly collected, for the persistent types in older stools tend to be the dye-resistant. If positive stools are not available inoculate fecal

*NOTE: The hemolytic streptococcus that we designate as "Type IIIa" has a definite etiological association. Our bacteriological findings in the past point to this organism as the principal causative agent in serious secondary infections. Other types of streptococci may be the cause of pathological conditions, however.

specimens with recently isolated cultures. Three to five fecal specimens should be selected to compensate for variability in fecal flora.

The test: Follow routine procedure of dilution and inoculation using two plates of each dye and one of Endo's medium for control with each set. Plates are read at the end of 24 and 48 hour incubation period. Two dilutions are selected: first, the amount that shows no reduction in size and number of typhoid colonies as compared with control, and marked inhibitions of fecal flora; second, amount that shows a moderate inhibition of typhoid colonies and a still greater inhibition of fecal flora.

Stains and Solutions

All stock bottles shall be labelled with formulae and labels protected by paraffin or collodion.

I. Loeffler's Methylene Blue.

Dissolve 1.0 gram potassium hydroxide in 100 mils of distilled water.

This makes a 1-100 stock solution of potassium hydroxide. Take 1.0 mil of the stock solution and add to 99.0 mils distilled water. This makes a 1-10,000 KOH solution. Add to the latter Methylene Blue—Sat. Alcoholic Solution.....30.0 mils. Shake and filter. Allow at least one week for aging and check new supply of stain before putting into use.

II. Differential Stains.

1. Modification of the Neisser's method for differentiating polar bodies. Films are stained in Neisser's blue 10-30 seconds. They are then washed in water and placed in Gram's iodine for 30 seconds.

Neisser's blue is made as follows: (M. Neisser—Zeitsebr.f.Hyg. 24:443, 1897.)

Methylene blue (1 gram dissolved in alcohol 96%) 20 mils.

Distilled water.....950 mils.

Glacial acetic acid..... 50 mils.

2. Epstein's stain. (Jr. Inf. Dis., 3:770, 1906.)
1% pyronin, or Loeffler's methylene blue for 30 seconds.
Wash. Gram's iodine 30 seconds.

3. Albert's stain. (Am. Jr. Public Health, 10:936, 1920.)

Solution I. Let stand one day and filter.

Toluidin blue—.15 gm. Methyl green—.20 gm.

Acetic acid—1.0 mils (Glacial). Alcohol 95%—2.0 mils.

Distilled water—100 mils.

Solution II.

Iodin—2.0 gm. Pot. Iodid—3.0 gm. Distilled water—300 mils.

Stain:

Solution I. 10 to 30 seconds.

Wash:

Solution II. 10 to 30 seconds.

Dry:

Examine.

4. Greenthal's stain. (Am. Jr. Dis. Child. 18:25. (July) 1919.
Crystal violet—0.07 gm. (Gruebler). Methylene blue—0.10 gm.
Glacial acetic acid—3.00 mils. Distilled water—1,000 mils.

III. Modification of Gram's Stain.

1. Aniline water gentian violet.

Distilled water—100.0 mils. Add 0.5 mil anilin and shake.

Filter and add saturated alcoholic solution gentian violet—10.0 mils. Filter.

NOTE: This solution will not keep longer than 14 days.

2. Lugol's iodine solution (Gram's).

Iodin—1.0 gm. Potassium iodid—3.0 gms. Dissolve in 25.0 mils distilled water by heating. Add solution to 275.0 mils distilled water. Shake and filter.

3. Ethyl alcohol 95%.

4. Safranin aqueous solution.

Safranin Y—1.0 gm. Distilled water—100.0 mils. Dissolve dye in distilled water and filter.

Method:

- (a) Cover smear after fixing with solution 1 for 30 seconds to one minute. (b) Wash. (c) Cover with solution 2 one minute. (d) Wash. (e) Cover with fresh solution 3 time according to character of smear. Thin culture preparations require 20 to 30 seconds. (f) Wash. (g) Cover with solution 4 for one minute. (h) Wash and dry.

IV. Ziehl-Neelson's stain for acid-fast organisms. (Neelson, Deut. Med. Woch. 1883.)

1. Carbol fuchsin. (Ziehl, Deut. Med. Woch. 1882.)

Basic fuchsin-saturated alcohol solution—10 mils. Carbolic acid 3.0% aqueous solution—90 mils. Filter.

2. Acid alcohol.

To 95% alcohol, add 3% HCl.

3. Counter stain.

(a) Aqueous methylene blue.

Methylene blue (Coleman and Bell for bacilli) — 1.5 gms.
Distilled water — 100.0 mls. Add water to dye in bottle,
shake occasionally. Filter after standing 48 hours.

(b) Loeffler's alkaline methylene blue.

Method:

- (a) Cover with 1. and steam five minutes. (b) Wash. (c)
Cover with 2. and allow to decolorize for five minutes.
(d) Wash. (e) Cover solution 3 for one minute. (f)
Wash and dry.

V. Hiss Capsile Stain. (Jr. Exp. Med. 6:317, 1905.)

1. Gentian violet—saturated alcoholic solution.

Gentian violet—5.0 gms. Place in stoppered bottle of about
200 mls capacity and add ethyl alcohol—100 mls. Allow
alcohol to remain in contact with the dye for two hours, shaking
vigorously every few minutes. Filter.

2. Saturated alcoholic solution gentian violet—5 mls. Distilled
water—95 mls.

3. Copper sulphate solution. Make up 20% solution CuSO_4 .

Method:

- (a) Flood smear with 1. and steam 30 seconds. (b) Wash off
immediately with 2. and blot. Do not use water at any
step. (Smears from cultures should be made in a drop of
serum.)

VI. Spirocheta silver stain. Modification of Fontana's method.

1. Ruge's fluid.

Acetic acid—1 mil. Formalin—20 mls. Distilled water—100
mls.

2. Mordant.

Tannic acid—5 gm. 1% carbolic acid solution—100 mls.

3. Silver stain. (Fontana's.)

Make $\frac{1}{4}\%$ solution silver nitrate in distilled water. Add
ammonia drop by drop until precipitate first formed begins to
dissolve and solution becomes slightly opalescent.

Method:

- (a) Flood smear with 1. several times. (b) Wash. (c) Flood
with mordant and steam for 30 seconds. (d) Wash with
distilled water. (e) Flood with 3. and steam lightly 30
seconds. (f) Wash and dry.

VII. Wright's Stain.

Dry Stain (Coleman Bell)—0.1 gm. Methyl alcohol (acetone-free) —100.0 mls. Place in stoppered bottle and allow to stand with occasional shaking for 24 hours. Filter.

Method:

1. Cover smear with Wright's stain and leave 30 seconds. 2. Dilute with an equal amount of distilled water and allow to act four minutes.
2. Wash in distilled water about 30 seconds and dry.

VIII. Giemsa's Blood Stain.

1. Stock solution.

Giemsa dry stain—0.3 gms. C. P. Glycerine—24 mls. C. P. methyl alcohol—25 mls. Allow to stand for 24 hours, shake occasionally and filter.

2. Dilute Stain.

Stock solution—1.0 mil. Distilled water—9.0 mil.

Method:

1. To fix: Flood film with methyl alcohol for 30 seconds. 2. To stain: Flood film with dilute stain for 15 minutes. 3. Rinse with distilled water and dry.

Examination of Feces for Intestinal Parasites

M. S. MARSHALL, Ph. D.

1. Read all information given with specimen when it comes in.
2. If local or near local (i.e. fresh specimen) and calling for protozoa examination, make as soon as possible.

If doctor calls for protozoa examination that is all that is necessary unless ova are observed during the examination for protozoa, or unless bacteriological examination is indicated by information given.

If doctor calls for ova examination look only for ova unless protozoa are noticed or bacteriological examination is indicated by the nature of the information given.

If doctor specifically requests parasite examination, look both for protozoa and ova, and make bacteriological examination if the nature of any information given would indicate.

If doctor does not specify examination to be made, as e.g. a case of stubborn undiagnosed chronic diarrhoea, make all examinations,—protozoa, ova, bacteriological. If any special examinations suggest themselves from observation of the specimen and from the information given, as fat examination, occult blood examination, tuberculosis examination, etc., make these.

3. Otherwise make examinations indicated as routine calls for during the day.
- (a) Protozoa: Saline fresh mount method, especially if fresh. Iodin-eosin wet stain method, especially if old.
 - (b) Ova: Direct wet mount, saline or water, mechanical stage examination of two or more cover glasses; or CaCl_2 concentration method; or Barber's concentration method; or washing method.
- Where both examinations are to be made, the protozoa slide may be examined for ova under low power. It is sometimes possible to find ova without concentration or extended search.

I. Protozoa:

For protozoa examinations, the second sample following a mild purge is recommended by many authors, and is not preferred by many others. A saline purge is preferable, being mild and not affecting any organism, whereas an oil purge is likely to be confusing in the examination. A saline purge consists of a large amount of salt solution (teaspoonful to a quart), warm, taken on an empty stomach.

If circumstances warrant, it is advisable to reserve report on protozoa until motion is observed. This is not, of course, possible with an old specimen, as living forms with the exception of the *Trichomonas* are very likely to be disintegrated within a few hours after the stool is passed, whereas the cysts may last several weeks.

Several Procedures follow:

1. Fresh Saline Mount: A small amount of feces is rubbed up in physiological saline, and examined under high power. The saline is imperative as a suspending fluid instead of water.
2. Live Stain: The saline suspension may be tinged with a little neutral red, 0.5% solution aqueous. The granules of amoebae and flagellates stain a striking rose pink, thus differentiating them from vegetable and human cells. If the dilution of the stain is great enough, the protozoa will not be killed. (Stitt, p. 64.)
3. Cyst Stain: Solution I consists of a saturated solution of KI, saturating saline with iodine crystals, and making in this a 5% solution of KI. Solution II is a saturated solution of eosin in normal saline. Solutions I and II are used in equal volume, fresh. Mixed they keep only 12 hours. A drop of the mixture is placed on a slide and a drop of saline next to it. The feces is emulsified in each, and a cover glass dropped on the two drops, causing them to run together. Fecal particles bacteria, yeasts (except larger forms) stain at once. Cysts appear as bright spherules, becoming yellow, and the glycogen filled vacuoles, if present, become light or dark brown. Nuclei are clearly defined as the iodine penetrates, in *Ent. coli* and *Ent. histolytica*, especially; with *End. nana* they can barely be distinguished. (Stitt, p. 65).

4. Concentration: One concentration method for protozoa consists of mixing with saline, and straining through wet cheesecloth. Centrifuge. Draw off supernatant fluid, and repeat, until supernatant fluid is clear. The cysts will be found in the bottom of the centrifuge tube. Another procedure is to strain into a tall cylinder graduate and allow to settle over night, examine the sediment which is obtained with a long pipette. (Hegner and Taliaferro, p. 492.)
5. Ether concentration: Add one gram or more of feces to 30 c.c. of saline and mix well. Add 5 c.c. of ether and stir well. Place quickly in a separatory funnel, allow to stand 5 to 7 minutes. The cysts will settle to the bottom of the saline, whereas the fine debris will float on the water just below the ether. Draw off 15 c.c. into centrifuge tube and centrifuge. Examine wet stained preparation of sediment. (Hegner and Taliaferro, p. 493.)
6. Permanent Mount: (Hegner and Taliaferro, p. 495.)
 - (a) Smear a cover glass, thinly, moist.
 - (b) Drop while moist into warm Schaudinn's fluid, warmed until steam rises. Schaudinn's fluid:
 Distilled water saturated with HgCl_2 65 parts
 95% alcohol..... 33 parts
 Glacial acetic acid..... 2 to 5 parts
 The film will float at first. After a few minutes it can be completely immersed and left 10 to 20 minutes.
 - (c) Wash and harden as follows:
 - (1) Wash in 50% alcohol few minutes.
 - (2) Wash in 70% alcohol to which few drops of alcoholic iodine solution has been added to remove all traces of HgCl_2 , for at least 10 minutes.
 - (3) Harden in 95% alcohol from few minutes to a few days. At least an hour preferable.
 - (4) Hydrate by successive immersions a few minutes each in 70% alcohol, 50% alcohol, and distilled water.
 - (d) Mordant by placing in 2% aqueous iron alum (ammonium ferric sulphate) from 30 minutes to over night; at least 2 hours preferable.
 - (e) Wash in distilled water a few seconds.
 - (f) Stain in 0.5% aqueous haematoxylin solution, ripe,* several hours; over night better.

*The ripening of the haematoxylin may be done by aging for several weeks. Or one may dissolve 0.1 gm. of stain in 3 to 10 c.c. of 95% to 100% alcohol, make up to 50 c.c. with 95% alcohol, and age:

- (1) Heating with K chlorate, or
- (2) Adding few drops of Dakin's solution, or
- (3) Adding few drops of chlorate of lime and adding K carbonate. Dash 45 c.c. distilled water into stain.

- (g) Wash in distilled water.
- (h) Differentiate in 1-2% aqueous iron alum solution. This demands care. Expose to strong solution until the decolorizing has reached near sufficiency. Then use weaker 0.5% solution. The process must be followed with microscopic examination. Wash in running tap water 20 minutes when done.
- (i) Wash in distilled water.
- (j) Dehydrate by 5 minute immersions in 50% alcohol, 70%, 95%, and absolute alcohol, two changes.
- (k) Clear by two changes in xylol, 5 minutes each.
- (l) Mount in Canada balsam.

II. Helminthes:

The remarks relative to purges obtain both for protozoa and for worm examinations.

A calibrated ocular micrometer disk is essential for parasites examinations, both protozoa and helminthes, in measuring sizes of live protozoa, cysts and ova.

Examinations for ova of parasitic worms, helminthes, in the feces are generally possible by direct microscopic examination with a wet mount. Both the inside and outside of a feces specimen should be examined if the specimen is not homogenous. This is even more necessary with protozoa than with helminthes. Examinations may also be made for adult and larval worms. Ova are present in the feces in infections with most worms, although with some, even in cases of infection, occurrence of ova in the feces is rare. *Taenia* ova might be especially mentioned as rarely showing ova in the feces in the early stages of infection.

1. Direct mount: Ova appear when mounted in saline or in water as yellowish or brownish well organized particles from 15 to 70 microns in length or diameter. A thicker suspension is to be preferred for ova examinations than for protozoa examinations, and the low power objective may be used. If several preparations are made and systematically examined with a mechanical stage, concentration methods are generally not demanded. It has been stated that direct examination will locate 40% of helminthe infections, concentration methods 55%, and cultural methods 99%. This is stated of hookworms only, however.
2. Washing method: Add small amount of feces to tap water in Wassermann vial, mix thoroughly, and strain rapidly into smaller vial through wet cheesecloth. Let stand 1 minute, decant quickly, and refill. Repeat until clear, and examine thick suspension of sediment. It is important to mix sediment thoroughly before taking specimen to examine, as ova stick to the glass and must be washed off and transferred to slide.

3. Barber's concentration method: Emulsify feces in equal parts of glycerine and saturated salt solution, and examine the top of the drop for ova. Or centrifuge in this solution, drop a little cotton on the surface of the centrifuged liquid, add 3 or 4 drops of melted agar, and remove and examine among the threads of cotton for ova. (Stitt, p. 466.)
4. Bass' method: Centrifuge feces specimen, add CaCl_2 sp. gr. 1.050 and centrifuge, draw off supernatant fluid and add calcium chloride sp. gr. 1.250 and centrifuge. This will bring the eggs to the top of the fluid. (Stitt, p. 466, Rivas p. 355.)
5. Lane's levitation method: Centrifuge feces and mix sediment with 1 c.c. of water. Allow to stand 5 minutes. Immerse in water and manipulate to remove the coarse material. Ova will stick to the slide. The whole preparation is made on a slide. (Stitt, p. 466.)
6. Cultural method, hookworms: It is probable that this method would be successful with other nematoda. Make a pile of filter paper circles 2 inches in diameter and a quarter of an inch high in a 4 inch Petri dish. Fill with water to the top of the paper. Spread a thick layer of feces on the filter paper pile. The larvae will be hatched in about 6 days, and will swim free in the surrounding water, where they may be located by centrifuging the water and examining. (Stitt, p. 467.)
7. Adult or larvae examination: The whole feces specimen may be rubbed up in tap water, allowed to settle a moment, rewashed any number of times to get a clear water. If this is done in a large half of a moist chamber, macroscopic examination is simple, and the worms will not be removed with the tap water as they are washed, but will stay in the bottom. The water must, of course, be poured off with some care.

Or the whole specimen may be washed through a fine screen, the lumps broken up and washed on through, the worms being left on the screen.

REFERENCES

1. Practical Bacteriology, Blood Work and Animal Parasitology, Stitt, 7th ed. Philadelphia, 1923.
2. Human Protozoology, Hegner and Taliaferro, New York, 1924.
3. Human Parasitology, Rivas, Philadelphia, 1920.

CHAPTER IX

OUTLINE OF METHODS OF MEDIA PRODUCTION AND GLASSWARE PREPARATION

M. S. MARSHALL, Ph.D.
LUCY DELL HENRY, B. S.

I. Glassware preparation. (Media Room)

1. No glassware accepted from washroom unless thoroughly cleaned and dried.
2. Pipettes. (Fig. 10.)
 - (a) Sorting—general bacteriology:

0.2 mil grad. 0.001.	2.0 mil grad. 0.01.
1.0 mil grad. 0.1.	5.0 mil grad. 0.1.
1.0 mil grad. 0.01.	10.0 mil grad. 0.1.
2.0 mil grad. 0.1.	
 - (b) Sorting,—water and milk: Non-graduated 1.0, 9.0, and 10.0 mil.
 - (c) Preparation for sterilization.
 - (1) All bacteriologic pipettes are plugged with non absorbent cotton, wrapped singly, and labeled with size and graduation.
 - (2) All water and milk pipettes sterilized in metal containers,—1.0 mil and 9 mil in round containers, and 10.0 mil in rectangular containers. Number in container not specified.

3. Petri dishes. (Fig. 17.)

- (a) Porous top type wrapped 6 to 8 in package.
- (b) Glass plates placed in metal containers.

II. Sterilization.

1. Hot air oven—glassware.

- (a) Petri dishes,—metal containers placed on lower shelf, wrapped plates on top shelf. Oven switch turned on to full, oven heated to 180° C. (1½ hours), switch turned to low, and oven held at 180° C. for 1 hour. Cooled slowly to 75° C. before opening.
 - (b) Pipettes,—wrapped pipettes are placed in wire basket on top shelf to avoid scorching paper, and treated as Petri dishes. Metal containers on any shelf.
- (2) Arnold sterilizer. Adjust water level before lighting gas or turning on steam.
- (a) Agars cooked for ¾ hour,—gas or steam then turned off to allow agar to filter (cf. III, 1, (d), (3)).
 - (b) Meat infusions heated until meat is thoroughly cooked.

(c) Infectious material.

- (1) Collected at 11:45 A. M. each day except Saturday from all bacteriologic rooms.
- (2) Heated at least 1 hour in live steam. Agar bottles emptied before placing on tray for washroom. Material ready for collection by 1:30 P.M.
- (3) Sputums for tuberculosis examination heated from 12 to 1 P. M. with contaminated material. Container must be placed so that no media or other liquid can enter sputum jars.

3. Autoclaves. Fill water tank at least $\frac{2}{3}$ full before lighting gas or turning on steam.

(a) Media. Stock or ordinary media heated 20 minutes at 15 lbs. Special media as ordered.

(b) Glassware.

- (1) Specimen jars (sputum, urine, etc.) heated 30 minutes at 15 lbs.
- (2) Keidel tubes, 30 minutes at 25 lbs.,—vacuum drawn after sterilization. Tubes are spread out on large trays during sterilization.
- (3) Mandler filters, 30 minutes at 20 lbs.,—cooled slowly in autoclaves. Wrapped in brown paper with filter and flask adjusted ready for use.

(c) Dressings.

- (1) Small packages, 30 minutes at 15 lbs.
- (2) Large packages and bags, 30 minutes at 20 lbs.
- (3) Marked "sterile" and dated before releasing.

4. Filters.

(a) Serums and sugars are sterilized by filtration through Mandler filters.

(b) Care of filters.

(1) Testing.

- a. New candles soaked 24 hours in distilled water.
- b. Distilled water drawn through pores of filter.
- c. Attached to air pressure, air turned on slowly until bubbles cover entire surface of candles. If no large bubbles appear, candle is probably all right.

(2) Cleaning.

- a. Candle brushed lightly.
- b. Covered with 0.5% aqueous solution of potassium permanganate.

- c. Attached to vacuum and solution drawn through.
- d. Permanganate rinsed out with distilled water.
- e. Covered with fresh 5% solution of sodium bisulphite.
- f. Rinsed by drawing distilled water through filter until filtrate shows no precipitate with barium chloride.

III. Media preparation,—general rules.

1. Procedures in preparation.

- (a) All formulae and methods of procedure are kept in card file.
- (b) Solutions.
 - (1) Dye and indicator solutions are made by bacteriologist in charge.
 - (2) Gillespie-Medalia standards are used for determining pH value. The standards are supplied by the Research Bacteriologist and kept in the refrigerator. Brom thymol blue or phenol red is used in making all routine pH adjustments.
 - (3) Sugars are weighed on analytical balance if 5 gms. or less are required. All sterile sugar filtrates are made in 20% solutions if possible.
 - (4) Standard acid and alkali solutions are made by the chemist.
 - (5) Phenol, 5% solution, is made under direct supervision of bacteriologist in charge. Rubber gloves must be worn throughout the process of making up the solution.
 - (6) Physiologic salt solution,—stock supply is kept in media room cupboard. Paper capped bottles and sputum jars of saline are kept in media room refrigerator for use in clinic room.
- (c) Broth.
 - (1) "Difco" peptone and "Difco" beef extract are used.
 - (2) Distilled water is always used.
 - (3) Broth is rendered sugar-free by treatment with B. coli communior for uses in semi-solid agar media to which definite carbohydrates are added.
- (d) Solid media.
 - (1) "Difco" agar is used.
 - (2) Distilled water is always used.

- (3) Filtering: 0.5% agar is filtered through filter paper; 1% to 2% agar is filtered with the use of vacuum bottles (Fig. 15); 3% Endo's infusion agar is filtered by the use of a Buchner funnel and vacuum pump.
- (4) Sterile filling: Blood agar slants, coagulated and non-coagulated, are poured aseptically directly from bottle to tube; semi-solid agar containing carbohydrates are poured aseptically directly from stock bottle to tube. Incubation 24 hours.
- (5) Beef serum for Loeffler's medium is ordered from Armour Fertilizer Co., Chicago, in 8 gallon lots. Grossly contaminated cans are discarded. Serum is filtered through Mandler filters into flasks, Labeled with lot number, pH (determined with phenol red), date received, and date filtered. Stored in refrigerator.

(e) Miscellaneous.

- (1) Special media. These are handled in accordance with the methods of the originators of such media, modified if called for in accordance with the use to which it is to be put. Detailed records are kept on file.
- (2) Sterile blood. Bleeding outfit is made (Fig. 14), sterilized, and cooled before using. Blood is shaken until defibrinated (10 minutes or more). A supply of defibrinated sterile sheep's and rabbit's blood is kept in the refrigerator in the media room at all times.

2. Standard cultures for checking media.

- (a) The bacteriologist in charge of media production is responsible for the care of standard cultures.

- (b) Sugar media and cultures used for checking:

Carbohydrates. Organisms used, and reaction.*

Dextrose	B. typhosus (706)+; B. morgani No. 1 (501) (+)
Lactose	B. typhosus (706)-; B. coli communior (601) (+)
Saccharose	B. coli communis (602) -; B. coli communior (601) (+)
Levulose	B. typhosus (706) +; B. morgani No. 1 (501) (+)

Maltose	B. morgani No. 1 (501) —; B. dysenteriae (Shiga) (1001) —; B. dysenteriae (Flexner) (1002) +
Mannite	As maltose.
Xylose	B. paratyphosus A (804) —; B. paratyphosus B (905) (+)
Salicin	B. typhosus (706) —; B. coli communis (602) (+)
Inulin	Strept. viridans —; pneumococcus +
Dulcitate	B. coli communior (601) (+); B. coli communis (602) (+); B. aerogenes (604) —
Adonite	B. coli communis (602) —; B. aerogenes (604) (+)
Inosite	B. coli communis (602) —; B. aerogenes (604) (+)
Triple sugars	B. coli (601) butt (+), slant (+); B. typhosus (706) butt +, slant —; B. paratyphosus A or B (804 or 905) butt (+), slant —

3. Labeling media for storage and distribution.

- (a) Cardboard key tags are used for labeling media. Media labeled with kind, date, and final pH.
- (b) Dyeing cotton plugs for sugar media. (Fig. 18.) All tubed media containing sugars except Krunwiede's triple sugar media and U-fermentation tubes are designated by colored plugs. No plugs are dyed until media has been properly checked for sterility, sugar reactions, and nutritive value. Each laboratory keeps a list of dyes used for the sugars and a list is posted in media room refrigerator.

Dextrose	red	Mannite	brown
Lactose	blue	Xylose	black
Saccharose	yellow	Levulose	purple
Maltose	salmon pink	Inulin	green

The more rarely used sugars are kept separate and labeled with tags.

4. Supplying media.

- (a) Minimum supply. Complete lists of minimum supply for media room and each division shall be filed in media room. (p. —). Each division shall post its minimum supply list on bulletin board.

* — = no acid or gas produced.

+ = acid produced.

(+) = acid and gas produced.

(000) = number of stock culture strain.

- (b) Daily rounds. In the morning the supplies of each division will be inspected by a media room technician. The supplies needed shall be delivered at that time.
- (c) Storage of media. Stock and special media will be kept in the refrigerator. (Fig. 16.) Lists will be found inside of door of refrigerators to designate place where each media is kept,—i.e., top shelf, sugars; second shelf, broth, etc.
- (d) Requisitions. Special requisition cards are used. No orders filled unless filed 24 hours or more before media is desired;—no orders filled on Saturday except in emergency.

5. Cleaning.

- (a) Daily routine. All equipment must be kept in proper place when not in use. Shelves, table-tops and sinks must be kept free from dust and foreign material and must be washed with phenol before sterile procedures.
- (b) Saturday morning. Except in emergency no media is made.
 - (1) Autoclaves. Inside washed with soap and water, shelves and supports removed and cleaned, outside cleaned with Puritan Metal Polish and polished with Bon Ami.
 - (2) Arnold. Outside cleaned with Puritan Metal Polish and then polished with Bon Ami; inside rack washed and lime removed from water container occasionally.
 - (3) Woodwork. Rubbed with damp cloth and dried.

IV. Records.

- 1. Formulae and procedures kept on cards in file.
- 2. Records kept on cards of kind and amount of media made. Amount prepared recorded in mls or liters except tubes of Loeffler's medium and plates, recorded by actual numbers; pH taken before and after sterilization, and final pH recorded.
- 3. Media shipped out recorded by date, kind, amount, and place; careful inspection made before releasing.
- 4. Dyes and indicators recorded by amount and date.
- 5. Contaminated media recorded by amount and date; included in monthly report.
- 6. Monthly reports made on first day of each month to cover production of previous month. Record kept of compiled data, and copy given to Director of Laboratories; details for preparation of report given in formula files.

Formulae for Media

I. Broth.

1. Beef extract broths.

(a) Plain beef extract broth.

Formula:

Distilled water.....	1000 mls
Peptone.....	10 gms.
Sodium chloride.....	5 gms.
Beef extract.....	3 gms.

Procedure:

(1) Mix materials and dissolve over free flame, (2) make up loss from evaporation, (3) adjust pH to 7.0 and boil 5 minutes, (4) filter through paper, (5) fill into French Squares, (6) sterilize 20 minutes at 15 lbs.

(b) Plain beef extract broth + dextrose (used for Loeffler's medium).

To plain beef extract broth which has been adjusted to pH 7.4 and sterilized, add aseptically 1% dextrose (5 c.c. of a sterile 20% solution per 100 c.c. broth).

(c) Sugar free extract broth.

(1) Inoculate beef extract broth with 24 hour broth culture of *B. coli communior* (2.5 c.c. broth culture per liter of broth), (2) incubate at 37° C. 24 hours, (3) boil broth in kettle for 10 minutes, (4) make up loss due to evaporation, (5) adjust to pH 7.0, (6) filter through paper, (7) sterilize 20 minutes at 15 lbs.

(d) Lactose salt free extract broth double strength (used for water analysis).

Formula:

Distilled water.....	1000 mls
Peptone.....	10 gms.
Beef extract.....	6 gms.

Procedure:

(1) Mix materials and dissolve over free flame, (2) make up loss due to evaporation, (3) adjust to pH 7.4, and boil 5 minutes, (4) filter through paper, (5) fill into 6x $\frac{3}{4}$ fermentation tubes, 10 mls each, (6) sterilize 20 minutes at 15 lbs, (7) add aseptically sufficient lactose to each tube to make 1.0% carbohydrate (0.5 mls sterile 20% lactose solution per tube), (8) incubate 37° C. for 24 hours to check sterility.

- (e) Lactose salt free extract broth, single strength (used for water analysis).

Formula:

Distilled water.....1000 mls
Peptone.....5 gms.
Beef extract.....3 gms.

Procedure:

- (1) Mix materials and dissolve over free flame, (2) make up loss due to evaporation, (3) adjust to pH 7.4 and boil 5 minutes, (4) filter through paper, (5) fill into 5x $\frac{5}{8}$ fermentation tubes, 5 mls each, (6) sterilize 20 minutes at 15 lbs., (7) add aseptically to each tube sufficient sterile solution of lactose to make 0.5% carbohydrate (0.175 mls 20% lactose solution per tube), (8) incubate 37° C. for 24 hours.

- (f) Lactose salt free broth + gentian violet (Hall and Ellefson, Jr. Bact. III, 1918, p. 329. Used for water analysis).

- (1) To 1000 mls of double strength lactose broth (see formula above) add 8.5 mls of 0.25% aqueous solution gentian violet, (2) tube in 5x $\frac{5}{8}$ fermentation tubes, 5 mls each, (3) sterilize 20 minutes at 15 lbs., (4) add aseptically to each tube sufficient sterile lactose solution to make 1% sugar (0.25 mls 20% lactose per tube).

- (g) Brilliant green lactose bile broth (Muer and Harris, Am. Jr. Pub. Health, 10:874, 1920.)

Formula:

Distilled water.....1000 mls
Ox gall (dried).....50 gms.
Peptone.....10 gms.

Procedure:

- (1) Mix materials and dissolve over free flame, (2) filter through paper, (3) to each liter of filtrate add 10 mls of a 1% solution of brilliant green, (4) fill into 5x $\frac{5}{8}$ fermentation tubes, 10 mls each, (5) sterilize 20 minutes at 15 lbs., (6) add aseptically to each tube sufficient sterile lactose solution to make 1% (0.5 mls 20% lactose per tube), (7) incubate 37° C. 24 hours.

- (h) Dicks' Toxin Broth.

Formula:

Distilled water.....1000 mls
Witte's peptone.....10 gms.
Liebig's meat extract.....3 gms.
Sodium chloride.....5 gms.

Defibrinated sheep's blood 2.5 mils.

Procedure:

- (1) Mix ingredients and dissolve over free flame, (2) make up loss due to evaporation, (3) adjust to pH 7.2, (4) sterilize 30 minutes at 15 lbs., (5) cool to 37° C. and add blood aseptically, (6) incubate 37° C., 24 hours.

2. Meat infusion.

(a) Plain meat infusion.

Meat, fat free, chopped 500 gms.
Distilled water 1000 mils.

Procedure:

- (1) Infuse in refrigerator over night, (2) infuse in Arnold steamer at 100° C. until meat is thoroughly cooked, (3) press broth out of meat, (4) cool and skim off fat, (5) make up to volume, (6) adjust to pH 7.0 and boil 5 minutes, (7) filter through filter paper, (8) store in 1500 mil amounts, (9) sterilize 20 minutes at 15 lbs.

(b) Plain meat infusion broth.

Formula:

Meat infusion 1000 mils
Peptone 10 gms.
Sodium chloride 5 gms.

Procedure:

- (1) Mix materials and dissolve over free flame, (2) make up loss due to evaporation, (3) adjust to pH 7.4 and boil 5 minutes, (4) filter through paper into flasks, (5) sterilize 20 minutes at 15 lbs.

(c) Beef infusion broth plus dextrose (used for blood culture).

Formula:

Beef infusion 1000 mils
Peptone 10 gms.
Sodium chloride 5 gms.

Procedure:

- (1) Mix materials and dissolve over free flame, (2) make up loss due to evaporation, (3) adjust to pH 7.4 and boil 5 minutes, (4) filter through paper, (5) fill into 5x $\frac{5}{8}$ tubes, 10 mils each, and in 100 and 200 mil flasks, 50 and 100 mils respectively, (6) sterilize 20 minutes at 15 lbs., (7) add aseptically sufficient sterile dextrose solution to make 1% dextrose (5 mils 20% solution per 100 mils broth), (8) incubate 37° C. for 48 hours.

- (d) Salt-free beef infusion broth (used for typhoid cultures).

Formula:

Beef infusion 1000 mls
Peptone 10 gms.

Procedure:

- (1) Mix materials and dissolve over free flame, (2) adjust to pH 7.0 and boil 5 minutes, (3) filter through paper, (4) fill into 5x $\frac{5}{8}$ tubes, 5 mls each, (5) sterilize 20 minutes at 15 lbs.

- (e) Phosphate beef infusion broth (used for streptococcus cultures).

- (1) Beef infusion broth 1000 mls. Adjust to pH 7.4.
(2) Phosphate mixture. Dissolve in 50 mls distilled water 23.8 gms. of Na_2HPO_4 and 2.1 gm. of KH_2PO_4 .
8 parts Na_2HPO_4 (23.8 gms. per 1 liter broth).
2 parts KH_2PO_4 (2.1 gms. per 1 liter broth).
(3) Add phosphate mixture to liter of broth. This is sufficient to make the broth isotonic.
(4) Filter through paper, (5) sterilize 20 min. at 15 lbs.

- (f) Toxin broth (used for diphtheria toxin production).

Formula:

Veal infusion 1000 mls
Witte's peptone 20 gms.
Sodium chloride 5 gms.

Procedure:

- (1) Mix materials and dissolve over free flame, (2) make up loss due to evaporation, (3) adjust to pH 7.0, (4) filter through paper, (5) fill into toxin flasks, 1 liter each, (6) sterilize 20 minutes at 15 lbs.

II. Agar.

1. Beef extract agar.

- (a) 2% agar plus sodium chloride (used for slants, deep tubes, triple sugars, brilliant green plates, and blood plates).

Formula:

Distilled water 1000 mls
Peptone 10 gms.
Beef extract 3 gms.
Sodium chloride 5 gms.
Agar 20 gms.

Procedure:

- (1) Mix materials and dissolve over free flame, (2) make up loss due to evaporation, (3) adjust to pH

7.0, (4) invert filtration bottles, (5) steam for 45 minutes, (6) cool to allow filtration, (7) fill into French Square bottles, 300 mls each, (8) sterilize 30 minutes at 15 lbs.

- (b) 1½% agar, salt free (used for water and milk analysis).

Formula:

Distilled water.....1000 mls
Peptone.....5 gms.
Beef extract.....3 gms.
Agar.....15 gms.

Procedure:

(1) Mix materials and dissolve over free flame, (2) make up loss due to evaporation, (3) adjust to pH 7.0, (4) invert filtration bottles, (5) steam for 45 min., (6) cool to allow filtration, (7) fill into French Square bottles, 100 and 200 mls each, (8) sterilize 30 minutes at 15 lbs.

- (c) 0.5% agar, sugar free semi-solid (used for carbohydrate media).

Formula:

Beef extract broth, sugar-free.....1000 mls
Agar.....5 gms.

Procedure:

(1) Mix materials and dissolve over free flame, (2) make up loss due to evaporation, (3) adjust to pH 7.2, (4) filter through paper, (5) fill into French Square bottles 100 and 200 mls each, (6) sterilize 20 minutes at 15 lbs.

- (d) Carbohydrate semi-solid agar (used for carbohydrate fermentation).

Formula:

Beef extract semi-solid agar, sugar free.....100 mls
Selected carbohydrate.....0.5%
Andrade indicator, amount as determined by titration (usually 1%).

Procedure:

(1) Melt the agar semi-solid, (2) add sterile filtered solution of sugar, (2.5 mls of 20% sugar solution per 100 mls of agar), (3) tube into 3½x1½ tubes about ⅓ full, (4) check with standard cultures, (5) incubate 37° C. 24 hours, (6) spray plugs after checks are determined to be all right.

- (e) Triple sugars (used for the detection of the typhoid colon group).

Formula:

2% agar beef extract, plus salt.....	1000 mls
Lactose.....	10 gms.
Saccharose.....	10 gms.
Dextrose.....	0.5 gms.
Andrade indicator, .5% solution, amount as determined by titration (usually 1%).	

Procedure:

- (1) Add sugars and Andrade indicator to melted agar, (2) adjust to pH 7.4, (3) fill two tubes with 10 c.c. each, (4) cool one and heat the other to boiling (the former should be colorless, the latter should be a deep pink,) (5) fill into 5x5/8 tubes, 3/8 full, (6) sterilize 15 minutes at 20 lbs., (7) check with standard cultures.

2. Meat infusion agar.

- (a) 1 1/2% beef infusion agar (used for slants and base media).

Formula:

Beef infusion.....	1000 mls
Peptone.....	10 gms.
Sodium chloride.....	5 gms.
Agar.....	15 gms.

Procedure:

- (1) Mix materials and dissolve agar over free flame, (2) make up loss due to evaporation, (3) adjust to pH 7.2, (4) invert filtration bottles, steam 45 minutes, (5) cool to allow filtration, (6) fill into 5x5/8 tubes and French Squares, 200 mls, (7) sterilize 20 minutes at 15 lbs.

- (b) Endo's infusion agar (used for typhoid and dysentery diagnosis).

Formula:

Beef infusion.....	1000 mls
Peptone.....	10 gms.
Sodium chloride.....	5 gms.
Agar.....	30 gms.

Procedure:

- (1) Mix materials and dissolve over free flame, (2) make up loss due to evaporation, (3) adjust reaction to pH 7.4, (4) steam for 45 minutes, (5) filter through

gauze and cotton pack in Buchner funnel with aid of vacuum pump, (6) fill into French Square bottles, 100 and 200 mil measured amounts, (7) sterilize 20 minutes at 15 lbs.

- (c) Beef infusion starch agar (used for gonococcus cultures).

Formula:

Beef infusion agar.....100 mils
Soluble starch.....1 gm.

Procedure:

(1) Melt agar, (2) adjust to pH 7.4 to 7.6, (3) add 1 gm. starch dissolved in cold distilled water, (4) steam 2 minutes, (5) fill into 5x $\frac{5}{8}$ tubes, (6) sterilize 20 minutes at 15 lbs., (7) slant.

- (d) Veal infusion glycerine agar (used for coagulated sheep's blood plates and diphtheria and tuberculosis stock cultures).

Formula:

Veal infusion.....1000 mils
Peptone.....10 gms.
Sodium chloride..... 5 gms.
Agar.....25 gms.
Glycerine.....5%.

Procedure:

(1) Mix all materials except glycerine and dissolve over free flame, (2) make up loss due to evaporation, (3) adjust to pH 7.6 to 7.8, (4) steam for 45 minutes, (5) filter through cotton gauze with Buchner funnel, (6) add 5% glycerine, (7) fill into French Square bottles, 100 mils each, (8) sterilize 20 minutes at 15 lbs.

- (e) Infusion semi-solid, 0.5% agar (used for stock cultures).

Formula:

Beef infusion.....1000 mils
Peptone.....10 gms.
Sodium chloride..... 5 gms.
Agar..... 5 gms.

Procedure:

(1) Mix materials and dissolve over free flame, (2) make up loss due to evaporation, (3) adjust to pH 7.2, (4) filter through gauze and cotton in glass funnel, (5) fill into French Square bottles, 100 and 200 mils each, (6) sterilize 20 minutes at 15 lbs.

3. Special solid media (used for gonococcus stock cultures).

(a) Beef heart blood agar.

Formula:

Distilled water.....	1000 mls
Beef heart (chopped).....	1 lb.
Peptone.....	10 gms.
Sodium chloride.....	5 gms.
Agar.....	30 gms.

Procedure:

(1) Mix all materials except agar and raise temp. to 70° C. slowly, stir constantly, hold at 70° C. 5 minutes, (2) press out meat, (3) add 3% agar and dissolve over free flame, (4) adjust to pH 7.4, (5) filter through cotton and gauze in Buchner funnel, (6) fill into containers, (7) sterilize 30 minutes at 15 lbs., (8) to the agar, cooled to about 50° C., add aseptically 25% horse blood, tube and slant, (9) incubate 37° C. 24 hours.

(b) Levine's agar used for eosin methylene blue plates in water analysis.

Formula:

Distilled water.....	1000 mls
Peptone.....	10 gms.
Dipotassium phosphate (K_2HPO_4).....	2 gms.
Agar.....	15 gms.

Procedure:

(1) Mix materials and dissolve over free flame, (2) make up loss due to evaporation, (3) invert filtration bottles, (4) steam for 45 minutes, (5) cool to allow filtration, (6) fill measured quantities into French Square bottles, 100 and 200 mls each, (7) sterilize 20 minutes at 15 lbs.

(c) Cooked meat media. (Holman, Jr. Bact. 4:149, 1919.) (Used especially for anaerobes.)

Formula:

Finely ground fresh lean beef.....	1 lb.
Distilled water.....	1000 mls

Procedure:

(1) Free meat from fat and gross fibers, (2) grind very fine, (3) add equal quantity of H_2O , (4) heat slowly to boiling point, stirring constantly, (5) adjust to pH 7.3 (as nearly as possible), (6) tube in 6x $\frac{3}{4}$ tubes, 2 inches deep, (7) sterilize 30 minutes at 10 lbs.

III. Plate media.

1. Endo's media (used for water analysis).

Formula:

Endo's base	100 mls
Lactose 20% solution	5 mls
Basic fuchsin, 5% alcoholic solution	0.5 mls
Sodium sulphite, fresh 10% solution	2.5 mls

Procedure:

(1) Melt Endo's base, (2) adjust to pH 7.4 with sterile 10% sodium carbonate solution, (3) add aseptically other ingredients, (4) mix thoroughly and pour into porous top plates, leaving covers ajar until solid.

2. Endo's media (used for typhoid-dysentery routine diagnosis).

Formula:

Endo's base	100 mls
Lactose 20% solution	5 mls
Basic fuchsin, 5 % alcoholic solution	0.2 mls
Sodium sulphite, fresh 10% solution	1.0 ml

Procedure:

(1) Melt Endo's base, (2) adjust to pH 7.4 with sterile 10% sodium carbonate solution, (3) add aseptically other ingredients, (4) mix thoroughly and pour into porous top plates, leaving covers ajar until solid.

Ref: Endo's Medium, C. C. Young and M. S. Marshall, Jr. Lab. and Clin. Med., 10:532, Apr., 1925.

3. Brilliant green agar (used for routine typhoid diagnosis).

Formula:

2% agar beef extract + sodium chloride	100 mls
20% lactose - 2% dextrose solution	5 mls
Andrade indicator, amount as determined by titration, Brilliant green solution 0.1%, amount as indicated by standardization test. (Usually 0.2 and 0.4 mls.)	

Procedure:

(1) Melt agar, (2) add aseptically other ingredients, (3) pour into porous cover plates, leaving top ajar until solid.

4. Sheep's blood agar (used for routine throat culture work).

Formula:

Agar beef extract + sodium chloride	100 mls
Sterile sheep's blood	5 mls

Procedure:

(1) Melt agar, (2) cool to 45° C., add blood aseptically, (3)

pour plates, (4) incubate in inverted position 24 hours at 37° C., (5) store in refrigerator.

5. Eosin methylene blue agar (used for typhoid diagnosis).

Formula:

2% agar plus sodium chloride.....	100 mls
20% lactose — 20% saccharose solution.....	2.5 mls
2% solution Eosin Y aqueous.....	2.0 mls
0.5% methylene blue aqueous.....	2.0 mls

Procedure:

(1) Melt agar, (2) add aseptically other ingredients, (3) mix thoroughly, (4) pour plates, leaving cover ajar until solid.

6. Levine's eosin methylene blue agar (used in water analysis).

Formula:

Levine's phosphate agar.....	100 mls
20% lactose solution.....	5 mls
3% eosin yellowish.....	2 mls
0.5% methylene blue.....	2 mls

Procedure:

(1) Melt agar, (2) add aseptically other ingredients, (3) mix thoroughly, (4) pour into porous top plates.

7. Ascitic fluid agar (used for gonococcus cultures).

Formula:

2% agar meat infusion.....	100 mls
Serum fluid.....	33 $\frac{1}{3}$ mls

Procedure:

(1) Melt agar infusion, (2) cool to 45° C., (3) add ascites fluid aseptically, (4) pour into glass top plates, (5) incubate 37° C., 24 hours. •

8. Little plate media. (Jr. A. M. A. Jan. 1, 1921.) (Used for diphtheria diagnosis.)

Formula:

- (a) 1 $\frac{1}{2}$ % agar beef infusion. Adjust to pH 7.4. Fill into 6x $\frac{3}{4}$ tubes, 3 mls each. Sterilize 20 minutes at 15 lbs.
- (b) Use unpasteurized milk. Allow cream to rise, and siphon off the milk. Fill into 5x $\frac{5}{8}$ tubes, 3 mls each. Sterilize 3 successive days in Arnold 20 minutes 100°.
- (c) Filtered serum—1 part. Sterile distilled water—3 parts. Fill into 3 $\frac{1}{2}$ x1 $\frac{1}{2}$ tubes, 3 mls each. Sterilize 3 successive days in Arnold 20 minutes 100°.

Procedure:

(1) Melt agar and cool to 45° C., (2) warm milk and serum mixture to 45° C., (3) add aseptically the milk and serum to the agar, (4) mix thoroughly and pour into plates.

9. Loeffler's plates (used for isolation of *B. diphtheriae*).

Formula:

Beef extract broth.....	100 mls
20% dextrose solution.....	5 mls
Filtered beef serum.....	50 mls

Procedure:

(1) To sterile broth add aseptically the dextrose solution and beef serum, (2) mix thoroughly, (3) pour into Petri dishes, (4) coagulate in autoclave. Close all valves and bring pressure slowly to 15 lbs. Hold for 20 minutes. Cool slowly. (5) Incubate 37° C. for 48 hours.

IV. Serum tube media.

1. Loeffler's serum slants (used for routine throat culture diagnosis).

Formula:

Filtered beef serum.....	3 parts
Broth beef extract + 1% dextrose.....	1 part

Procedure:

(1) Mix serum with broth and dextrose solution, (2) adjust to pH 7.2 — 7.4, (3) use sterile tubing apparatus and fill into 3½x½ tubes, 4 mls each, (4) place in slant baskets, long slant, short butt, (5) coagulate and sterilize in autoclave in one operation. Bring pressure in autoclave up to 15 lbs. in 1-1½ hours and hold for 30 minutes. Release pressure very slowly. Incubate sample from each slant rack 37° C. for 48 hours. Store remainder of tubes in refrigerator until sterility has been determined. Tubes are then ready for use.

Caution: It is very important that the valves be steam tight on the autoclaves and that the pressure is raised and lowered slowly, otherwise the slant will be broken up.

2. Hiss serum water. (Jr. Exp. Med. 6:317, 1905.) (Used for differentiation of pneumococcus and streptococcus.)

Formula:

Filtered beef serum.....	1 part
Distilled water.....	2½ parts
Litmus.....	5% solution to make deep blue
Inulin.....	5%

Procedure:

- (1) Mix serum and water, (2) heat in Arnold 100° C. for 15 minutes, (3) add litmus to make deep blue color, (4) add sugar, dry, (5) fill as ordered, (6) sterilize in Arnold for three successive days at 100° C, for 20 minutes.

V. Miscellaneous media.

1. Egg media, a modification of Petroff's method (used for B. tuberculosis cultures or other acid-fast organisms).

Formula:

- Beef infusion broth + 15% glycerine.....1 part
Adjust to pH 7.4 and sterilize 20 minutes at 15 lbs.
Eggs, very fresh.....2 parts.

Procedure:

- (1) Wash eggs in lukewarm water and then in 1% carbolic acid, (2) dry on clean filter paper, (3) chip shell at large end with flamed forceps, (4) remove shell so that membrane is exposed about the size of a dime, (5) pierce other end of egg with flamed forceps so that contents of egg may be blown out of the shell, (6) blow egg sterily into sterile container with beads, (7) weigh egg and add the broth (1:2) or 250 mils to six eggs, (8) If dye is needed add 1 mil of 1% alcoholic solution gentian violet to 100 mils of media, (9) fill sterily in 5x $\frac{5}{8}$ tubes and slant in racks, (10) coagulate and sterilize in autoclave. Bring pressure in autoclave up to 15 lbs. in 1-1 $\frac{1}{2}$ hours and hold for 30 minutes. Release pressure slowly.

2. Gelatin (used for stab cultures).

Formula:

- Distilled water.....1000 mils
Peptone..... 10 gms.
Beef extract..... 3 gms.
Gelatin.....150 gms.

Procedure:

- (1) Mix materials and dissolve in water bath, (2) adjust to pH 7.2 and boil 5 minutes, (3) invert filtration bottles, (4) cook in Arnold one hour and allow to filter, (5) fill as ordered, (6) sterilize 20 minutes at 15 lbs., (7) keep in refrigerator to maintain solidity.

3. Litmus milk.

Formula:

- Fresh, non-pasteurized milk.....500 mils
Litmus indicator to make deep blue.

Procedure:

- (1) Allow cream to rise, (2) siphon off the milk, (3) measure the milk and add litmus indicator, (4) reaction should be slightly alkaline to litmus, (5) tube as ordered, (6) sterilize in Arnold three successive days at 100° C. for 20 minutes.

VII. Solutions.

1. Phenol solution (used for disinfectant).

Formula:

Phenol crystals.....	50 mls
Distilled water, volume to.....	1000 mls

Procedure:

- (1) Melt crystals in steam and measure into graduate, (2) add the water hot and mix thoroughly.

2. Physiological salt solution (used for bacteriological dilutions).

Formula:

Sodium chloride.....	8.5 gms.
Distilled water, volume to.....	1000 mls

Procedure:

- (1) Mix materials and dissolve thoroughly, (2) filter through paper, (3) fill into French Square bottles, 100 mls each, (4) sterilize 20 minutes 15 lbs.

3. Ringer's solution, modified (used for washing organisms).

Formula:

Distilled water.....	1000 mls
Sodium chloride.....	5 gms.
Potassium chloride.....	0.2 gms.
Calcium chloride.....	0.2 gms.
Sodium bicarbonate.....	.03 gms.
Lactose.....	1 gm.

Procedure:

- (1) Mix materials and dissolve thoroughly, (2) fill into French Square bottles, 100 mill amounts, (3) sterilize 20 minutes at 15 lbs.

4. Peptone solution for methyl red test (used in differentiation of fecal and non-fecal members of B. coli group).

Formula:

Distilled water.....	1000 mls
Peptone.....	5 gms.
Dipotassium hydrogen phosphate.....	5 gms.
Dextrose.....	5 gms.

Procedure:

- (1) Mix materials and dissolve over free flame, (2) make up volume and filter, (3) fill into 5x⁵/₈ tubes, 10-15 mls each, (4) sterilize 20 minutes at 15 lbs.

5. Dunham's peptone solution (used for dilution of feces in typhoid diagnosis).

Formula:

Distilled water.....	1000 mls
Peptone.....	10 gms.
Sodium chloride.....	5 gms.

Procedure:

- (1) Mix materials and dissolve over free flame, (2) filter through paper, (3) fill into French Squares, 100 mls each, (4) sterilize 20 minutes at 15 lbs.

6. Sugar solutions.

- (a) Lactose-dextrose solution (used for brilliant green plates).

Formula:

Lactose.....	20 gms.
Dextrose.....	2 gms.
Make up to 100 mls.	

Procedure:

- (1) Dissolve sugars, (2) filter through Mandler filter, (3) fill into 100 ml flasks, (4) test each flask for sterility, by transferring 1 ml of sugar solution to tube of beef infusion broth and incubating for 48 hours.

- (b) Single sugar solutions.

All sugar solutions are made up to 20% solutions when the solubility will permit and are sterilized by filtration. Each flask is tested separately for sterility.

7. Cleaning solutions.

- (a) Potassium permanganate (used for cleaning Mandler filters).

Formula:

Distilled water.....	1000 mls
Potassium permanganate.....	5 gms.
Dissolve the KMnO_4 in water and filter through paper.	

- (b) Sodium bisulphite (used for cleaning Mandler filters).

Formula:

Distilled water.....	1000 mls
Sodium bisulphite.....	50 gms.
Dissolve the NaHSO_3 in water and filter through paper.	

- (c) Dichromic acid.

Formula:

Conc. sulphuric acid.....	460 gms.
Water.....	300 gms.
Sodium dichromate.....	60 gms.

Procedure:

(1) Mix water and $\text{Na}_2\text{Cr}_2\text{O}_7$ and dissolve, (2) add H_2SO_4 *very* slowly, stirring constantly until the $\text{Na}_2\text{Cr}_2\text{O}_7$ is reprecipitated.

Notice: Great caution must be taken in adding the sulphuric acid to the sodium dichromate solution. The solution becomes intensely hot and may boil over.

VII. Dyes and indicators.

1. Andrade indicator. (Jr. Infect. Dis., 15:227, 1914).

Formula:

Distilled water.....100 mls
Acid fuchsin.....0.5 gms.

Procedure:

(1) Dissolve fuchsin in water, (2) decolorize with N/I NaOH., (3) filter through paper. Caution: Do not over decolorize. Keep in dark bottles.

2. Brom thymol blue. Gillespie-Medalia standards used. (Used in the determination of pH. of media.)

Formula:

Stock solution .2% alcoholic.

Test solution of .02%. Take 20 mls stock solution and make up to 200 mls with distilled water.

N. B. The standards and solution for testing are controlled by the Research Bacteriologist.

3. Litmus indicator. Prepare just before using.

Formula:

Merck's litmus.....5 gms.
Distilled water.....100 mls

Procedure:

(1) Mix materials, (2) steam in the Arnold two hours, shaking frequently, (3) filter through paper.

4. Phenol red. Gillespie-Medalia standards used. (Used for titrations of media.)

Formula:

Stock solution .2% alcoholic.

Test solution of 0.02%; take 20 mls stock solution and make up to 200 mls with distilled water.

5. Methyl red. Gillespie-Medalia standards used. (Used for titrations of media.)

Formula:

Stock solution 0.2% alcoholic.

Test solution 0.02%; take 20 mls stock solution and dilute to 200 mls with distilled water.

6. Alcoholic solution gentian violet. (Used for Petroff's egg media.)

Formula:

Gentian violet.....1.0 gms.
Methyl alcohol, volume to.....100 mils

Procedure:

(1) Pour part of the alcohol upon the dye, (2) stir and add remainder of the alcohol, (3) filter through paper.

7. Aqueous gentian violet. (Used for lactose broth in water analysis.)

Formula:

Distilled water.....100 mils
Gentian violet.....0.25 gms.

Procedure:

(1) Dissolve gentian violet in water, (2) filter through paper.

8. Aqueous methylene blue. (Used for eosin methylene blue plates.)

Formula:

Methylene blue.....0.5 gms.
Sterile distilled water, volume to.....100 mils

Procedure:

(1) Mix materials and shake well, (2) filter through paper.

9. Aqueous eosin 3%. (Used for eosin methylene blue plates.)

Formula:

Eosin yellowish.....3 gms.
Sterile distilled water.....100 mils

Procedure:

(1) Mix dye with the water, (2) filter through paper. Sterilization in autoclave 20 minutes at 15 lbs. is sometimes necessary.

10. Brilliant green. (Used for brilliant green plates.)

Formula:

Brilliant green dye.....0.1 gms.
Distilled water, volume to.....100 mils

Procedure:

(1) Mix dye and water in a flask, (2) filter through paper into a dark glass bottle. Caution: Discard the solution after one month, as it begins to deteriorate after that time.

11. Basic fuchsin. (Used for Endo plates.)

Formula:

Basic fuchsin.....5 gms.
Ethyl alcohol 95%, make up to 100 mils volume.

Procedure:

(1) Mix materials, (2) allow to stand several hours, shaking at intervals, (3) filter through paper. Solution must be saturated.

Media Base Supplies in Media Room

The following list of media will constitute the base supplies of the Media Division. These supplies are stored in the media room for daily distribution.

Kind of Media.	Minimum Amount on hand.	No.	Storage Container.	Amount in Container.
Infusion:				
Beef infusion.....	3 liters....	2	Bottles.....	1,500 mls
Broths:				
Beef extract.....	2 liters....	10	Fr. Sq.....	1-200 mls
Beef extract+dextrose.....	3 liters....	2	Bottles.....	1,500 mls
Beef extract, sugar free.....	3 liters....	2	Bottles.....	1,500 mls
Beef extract, lactose, sin. str.....		200	5 x $\frac{3}{8}$ tube.....	5 mls
Beef extract, lactose, dou. str.....		300	6 x $\frac{3}{8}$ tube.....	10 mls
Beef infusion.....	3 liters....	10	Fr. Sq.....	300 mls
		10	Flasks.....	50 mls
Beef infusion+dextrose.....	2 liters....	5	Flasks.....	100 mls
		30	5 x $\frac{3}{8}$ tube.....	10 mls
Beef infusion, salt-free.....	1 liter....	20	Fr. Sq.....	300 mls
		20	5 x $\frac{3}{8}$ tube.....	10 mls
Beef infusion phosphate.....	1 liter....	10	Flasks.....	100 mls
Veal infusion, toxin.....	1 liter....	3	Fr. Sq.....	300 mls
Agars:				
Beef extract+sodium chloride.....	2 liters....	7	Fr. Sq.....	300 mls
Beef extract, salt-free.....	2 liters....	10	Fr. Sq.....	1-200 mls
Beef extract semi-solid.....	1 liter....	5	Fr. Sq.....	200 mls
Beef extract semi-solid, sugar-free.....	1 liter....	10	Fr. Sq.....	100 mls
Beef infusion+sodium chloride.....	2 liters....	10	Fr. Sq.....	200 mls
Beef infusion, Endo's base.....	1 liter....	8	Fr. Sq.....	1-200 mls
Beef infusion semi-solid.....	1 liter....	10	Fr. Sq.....	200 mls
Special solid media:				
Levine's agar used for eosin methylene blue plate.....	1 liter....	5	Fr. Sq.....	200 mls
Dicks' agar.....	$\frac{1}{2}$ liter....	6	Fr. Sq.....	75 mls
Cooked meat media.....		30	6 x $\frac{3}{8}$ tube.....	15 mls
Tubed media:				
Triple sugars.....		100	5 x $\frac{3}{8}$ tube.....	12 mls
Dextrose, lactose, saccharose, levulose, maltose, mannite, and xylose semi-solid.....		25	3 $\frac{1}{2}$ x $\frac{1}{2}$ tube.....	
Beef extract slants.....		25	5 x $\frac{3}{8}$ tube.....	
Beef infusion slants.....		25	5 x $\frac{3}{8}$ tube.....	
Coagulated blood slants.....		40	5 x $\frac{3}{8}$ tube.....	
Beef infusion broth.....		25	5 x $\frac{3}{8}$ tube.....	
Beef infusion broth+dextrose.....		20	5 x $\frac{3}{8}$ tube.....	
Beef infusion broth, salt-free.....		20	5 x $\frac{3}{8}$ tube.....	
Peptone solution (for methyl red test).....		20	5 x $\frac{3}{8}$ tube.....	
Egg media.....		25	5 x $\frac{3}{8}$ tube.....	
Loeffler's slants.....		2,000	3 $\frac{1}{2}$ x $\frac{1}{2}$ tube.....	
Litmus milk.....		50	5 x $\frac{3}{8}$ tube.....	
Solutions:				
Phenol 5%.....	10 liters....			
Physiological salt.....	2 liters....	20	Fr. Sq.....	100 mls
Ringer's.....	$\frac{1}{2}$ liter....	5	Fr. Sq.....	100 mls
Peptone solution (Dunham's).....	1 liter....	5	Fr. Sq.....	200 mls
Sugars: (all 20% solutions) Dextrose, lactose, saccharose, levulose, maltose, mannite, and xylose.....	200 mls....	2	Flasks.....	100 mls
20% lactose-2% dextrose.....	100 mls....	1	Flasks.....	100 mls
Cleaning solutions:				
Potassium permanganate.....	1 liter....	1	Bottle.....	
Sodium bisulphite.....	1 liter....	1	Bottle.....	
Dichromic acid.....	4 liters....	1	Jar.....	
Dyes and Indicators:				
Andrade.....				
Brom thymol blue 0.02%.....				
Phenol red 0.02%.....				
Methyl red 0.02%.....				
Aqueous gentian violet 0.5%.....				
Aqueous methylene blue 0.5%.....				
Aqueous eosin yellowish 3%.....				
Brilliant green 0.1%.....				
Basic fuchsin, saturated alcoholic solution.....				

Glassware Preparation and Supply

- I. Special glassware: Wash all special glassware in the laboratories in which it is used.
- II. Preparation of routine glassware for collection.
 1. General rules.
 - (a) Non-infectious material.
 - (1) Put on trays provided for that purpose. All material not intended for the wash room must be kept off the trays.
 - (2) Place glass slides in jars provided especially for them.
 - (3) No glassware is accepted for washing which contains milk, blood or serum (cf. II. 1. (b) (3)).
 - (b) Infectious material.
 - (1) General: Place all contaminated material except Loeffler's medium and liquids in containers provided for that purpose.
 - (2) Loeffler's medium: Pack in small wire baskets ready for the sodium hydroxide tank.
 - (3) Fluids: Disinfect with 5% phenol, empty down drain, and place container on trays.
 2. Rules for individual laboratories.
 - (a) Water and milk.
 - (1) Tie glass stoppers on specimen bottles before sending to wash room.
 - (2) Rinse milk pipettes with water immediately after using.
 - (b) Clinical pathology.
 - (1) Empty specimens down drain.
 - (2) Tie glass stoppers on specimen bottles before sending to wash room.
 - (3) Rinse blood and serum out of tubes before placing on trays.
 - (c) Bacteriology, Nos. I and II.
 - (1) Rinse blood and serum from tubes and pipettes before putting on trays.
 - (2) Place pipettes and agglutination tubes in phenol jars.
 - (3) Fill sputum and feces jars with phenol and allow to stand over night.

(d) Serology.

- (1) Place pipettes, vials and tubes in separate containers and fill container with water immediately after using.

(e) Media.

- (1) Sterilize contaminated glassware from other laboratories.
- (2) Empty melted agar from bottles.

III. Collection of glassware. 8:30 a.m. and 1:30 p.m.

1. Replace trays and containers after emptying the used glassware.
2. Empty all trays and containers of used glassware in all diagnostic laboratories.
3. Empty phenol jars containing agglutination tubes.
4. Empty jars containing used glass slides.

IV. Cleaning glassware.

1. Sorting, washing and drying.

- (a) Petri dishes: Remove media, place edge down in dish pan keeping tops and bottoms separated, boil 15 minutes in soap solution, rinse twice in soft and twice in distilled water.
- (b) Large tubes containing media: Remove media, boil 15 minutes in soap solution, wash and brush tubes inside with hot soap water, rinse in soft water, place in commercial 10% HCl for two hours, rinse twice in soft water and twice in distilled water, pack inverted in wire baskets and dry.
- (c) Serology and agglutination tubes: Wash blood and serum out, boil 15 minutes in soap solution, rinse in soft water, place in cleaning solution* over night, rinse three times in soft water and twice in distilled water, pack inverted in wire baskets and dry.
- (d) Serology vials: Brush blood and serum out, boil 15 minutes in soap solution, brush inside of each vial while in the soap, rinse in soft water, place in commercial 10% HCl for few hours, rinse twice in soft water and twice in distilled water, pack inverted in wire baskets and dry.
- (e) Serology pipettes: (bacteriology pipettes are washed by media room technician). Force soft water through pipettes, place in cleaning solution over night, rinse twice in soft water and twice in distilled water, place in wire baskets and dry.

* Cleaning solution—sulphuric acid and sodium dichromate solution.

- (f) Slides: Place in xylol to remove oil, boil in soap solution 15 minutes, scour smears off with cleanser, rinse with soft water, drain, place in 70% alcohol and wipe dry with clean dry cloth.
- (g) Miscellaneous: (Flasks, bottles, etc.) Brush inside with hot soap solution, rinse twice in soft and twice in distilled water (care taken to pick out any clouded pieces and place in cleaning solution over night), invert in wire baskets and dry.
- (h) New glassware.
 - (1) Tubes and vials: Place in 10% HCl for 4 hours, rinse twice in soft and twice in distilled water, pack inverted in wire baskets and dry.
 - (2) Petri dishes: Brush and wash in hot soap solution, rinse twice in soft water and twice in distilled water, invert in wire baskets and dry.
 - (3) Slides and cover slips: Place in commercial 10% HCl for 2 hours, rinse in soft water, place in 70% alcohol, wipe each slide and cover slip dry with clean dry cloth.
 - (4) Pipettes: Place in cleaning solution 4 hours, rinse twice in soft water and twice in distilled water, place in wire basket and dry.
 - (5) Flasks, bottles and miscellaneous: Wash and brush in hot soap solution, rinse twice in soft and twice in distilled water.
- (i) All drying is done in hot air oven.

2. Distribution of clean glassware.

- (a) Return all clean glassware except serology tubes, vials, and pipettes which are delivered to the serology laboratory, to the supply room on the mezzanine floor.

V. Sterilization.

1. Preparation of glassware for sterilization.

- (a) Plain and fermentation tubes and tubes containing throat swabs, plug with non-absorbent cotton.
- (b) Flasks (Erlenmeyer and toxin), plug with gauze and non-absorbent cotton.
- (c) Water and urine bottles, place slip of paper between glass stopper and bottle and tie on paper cover.
- (d) Serology vials, put cork stopper in firmly.

- (e) Sputum jars, place rubber inserts in metal caps and snap on jars.
- (f) Vials used for tubing vaccines and diphtheria toxin-antitoxin, invert while moist into copper containers lined with gauze, and replace copper cover.

2. Sterilization.

- (a) Sterilized in glassware preparation room:

Plain and fermentation tubes, swabs, flasks, serology vials: Place in cold oven, turn switch to full heat, bring temperature to 160 C., turn switch to low and hold at that temperature for 1 hour, cool oven to 75 C. before opening door.

- (b) Sterilized in media room:

- (1) Bacteriological pipettes and Petri dishes in hot air oven.
- (2) Water and urine specimen bottles and sputum jars, sterilize in autoclave at 20 lbs. for 30 minutes.
- (3) Vaccine and toxin-antitoxin vials, sterilize in the autoclave at 20 lbs. for 60 minutes.

VI. Distribution of sterile glassware.

1. Inspect glassware supply in each laboratory daily at 8:30 a.m. Restock supply at that time.
2. Supplies in each laboratory kept up automatically.

6x $\frac{3}{4}$ plain tubes	36 Sanitary Bacteriology,
5x $\frac{5}{8}$ plain tubes	36 Research,
50 mil centrifuge tubes	10 Bacteriology No. II,
	10 Research,
15 mil centrifuge tubes	10 Bacteriology No. I,
	10 Bacteriology No. II,
	10 Research,
Agglutination tubes	500 Bacteriology No. I,
	100 Bacteriology No. II,
	100 Research,
Concave slides	50 Bacteriology No. I,
Special feces jars	20 Bacteriology No. I,
Glass slides	Fill jars provided for such in all
	laboratories. Note: New slides
	only in Bacteriology No. I.

VII. Minimum supplies of glassware constantly in stock (totals).

1. Sterile glassware:

Serology vials.....	5000	
Test tubes $3\frac{1}{2} \times 1\frac{1}{2}$	8000	
Test tubes $5 \times \frac{5}{8}$	1000	
Test tubes $6 \times \frac{3}{4}$	1000	
Swabs.....	10000	
Sputum jars.....	2000	
Water and urine bottles..	300	
Centrifuge tubes 50 mil..	50	
Centrifuge tubes 10 mil..	100	
Toxin flasks.....	50	
Erlenmeyer flasks.....	20-50	(sizes ranging from 100 mls to 3 liters)
Pipettes, 10 mil.....	100	
Pipettes, 5 mil.....	50	
Pipettes, 2 mil.....	50	
Pipettes, 1 mil.....	150	
Pipettes, 0.2 mil.....	600	

2. Clean but not sterile glassware:

French Squares.....	600	(sizes ranging from 6 to 16 ounces)
Test tubes (all sizes)....	10000	
Sputum jars.....	200	
Flasks (all sizes).....	100	
Bottles, glass stopper,		
2 liter.....	25	
Petri dishes.....	200	

Orders for Containers

Orders for containers which have accumulated during the preceding day will be shipped at 8 a.m. by the Glassware Preparation Division. Labels giving the physician's name and address, and the number and kind of containers sent will be typed. This information will be recorded in a book reserved for that purpose. Requests for containers will be filed by the Glassware Preparation Division and kept for three months.

Orders for:

Wassermann

- (1) One vial in each container. (2) Wassermann blank for each vial.
- (3) Return label.

Throat Swabs

- (1) Two swabs in each container. (2) Blue blank for each swab.
- (3) Return label.

Water and Urine Bottles

- (1) One bottle in each container. Water blank for bacteriological analysis of water, or (2) One bottle in each container. Urine blank. (3) Return label.

Sputum Bottle

- (1) One bottle in each container. (2) Sputum and typhoid blank for each bottle. (3) Return label.

Glass Slides

- (1) Number of slides to be sent will be stated on order blank.
- (2) One gonorrhea and one hematology blank for each slide.
- (3) Return label.

Aluminum Plates for Widal

- (1) One plate in each envelope. (2) One typhoid blank.
- (3) Return label.

Blood Chemistry

- (1) One vial containing preservative in each container.
- (2) Blood chemistry blank. (3) Return label.

CHAPTER X

OUTLINE OF METHODS OF PROCEDURE IN CLINICAL PATHOLOGY

A. B. HAW, M. S., *Physiological Chemist*

- I. The following blood examinations are made upon request if character of specimen permits.
 1. Cytology
 - (a) White cell count
 - (b) Red cell count
 2. Slide preparations for differential count, for examination for malarial parasites, identification of pathological leucocytes and pathological erythrocytes.
 3. Coagulation time, Sabroze's method. Stitt, p. 307.
 4. Hemoglobin determination
 - (a) Sahli method, or Dare's method.
 5. Blood grouping
 - (a) Jansky's classification
 - (b) Compatibility test is recommended when possible
 6. Chemistry
 - (a) Removal of proteins. (Ref.: Hawk, p. 276. Folin, p. 229)
 - (1) The protein-free blood filtrates are obtained by using tungstic acid.
 - (b) Non-protein nitrogen. (Ref.: Hawk, p. 277. Folin, p. 235)
 - (1) A micro-Kjeldahl is run on 5 cc. of blood filtrate and the ammonia formed is determined colorimetrically by direct nesslerization of the digestive mixture.
 - (c) Urea. (Ref.: Hawk, p. 278. Folin, p. 239)
 - (1) The urea is decomposed by means of the enzyme urease, the ammonia is either distilled or areated off and determined colorimetrically after nesslerization.
 - (d) Uric acid. (Ref.: Jr. Biol. Chem. 51:187, 1922. Myers, p. 61.)
 - (1) Benedict's new method for uric acid is applied directly to 5 c.c. of blood filtrate, Folin's new formaldehyde standards being used. (Jr. Biol. Chem., 54:153, 1922.)
 - (1) Ten cc. of blood filtrate are treated with an alkaline picrate solution and the color developed compared

in a colorimeter with that developed in a standard creatinine solution treated in the same way.

(f) Glucose. (Ref.: Hawk, p. 283. Folin, p. 253)

(1) The blood filtrate is heated with alkaline copper solution. The cuprous oxide formed is treated with a molybdate phosphate solution. The blue color obtained is compared in a colorimeter with that obtained from a standard glucose solution.

(g) Chlorides. (Ref.: Hawk, p. 285. Folin, p. 287)

(1) The chlorides are precipitated from the blood filtrate by means of a silver nitrate and the excess silver titrated with standard sulphocyanate solution using ferric ammonium sulphate as an indicator.

(h) Cholesterol. (Ref.: Hawk, p. 291)

(1) The blood is dried with plaster of Paris and extracted with chloroform. The cholesterol is then determined colorimetrically after adding to the chloroform extract, acetic anhydride and sulphuric acid.

(i) "Alkali reserve"

(1) Titration method. (Ref.: Hawk, p. 318)

Plasma is treated with an excess of standard acid which is titrated back with standard alkali under carefully standardized conditions.

(2) Direct method. (Ref.: Hawk, p. 311) (Myers p. 112.)

A known quantity of plasma is saturated with carbon dioxide acidified within a Van Slyke pipette and the carbon dioxide is liberated by the production of a partial vacuum. The liberated carbon dioxide is then placed under atmospheric pressure, its volume carefully measured and the volume correspondingly to 100 cc. of plasma calculated.

(j) Acetone bodies. (Ref.: War Manual No. 6, p. 107.) (Myers p. 174.)

(1) The acetone bodies in the protein-free blood filtrates are changed to free acetone and then precipitated by mercuric sulphate, the precipitate being filtered off and weighed.

References: Hawk's Practical Physiological Chemistry, 7th edition, 1921; Folin's Laboratory Manual, 3rd edition, 1922.

Myers' Practical Chemical Analysis of Blood. 2nd Edition. 1924.

II. Cerebro-Spinal Fluid.

1. Cell count to determine approximate number of cells in a c.mm., fluid should be fresh, but 6 to 8 hours will not change the cell count.
 - (a) Draw gentian violet tinged 10% acetic acid to mark 0.5 and the cerebrospinal fluid to 11. Count 9 sq. mm. and add $\frac{1}{6}$ of the number. This procedure does not apply to purulent fluid. Two to ten is a normal cell count.
2. Globulin
 - (a) Nonne test for globulin, also Ross-Jones method.
 - (1) Equal parts supersaturated ammonium sulphate and spinal fluid. A white cloud or opalescence in three min. means an increase of globulin. Recently the Ross-Jones ring has been employed. A white or grey ring at the point of contact of the two liquids is a positive reaction.
 - (b) Pandy's reaction
 - (1) One drop of spinal fluid in .2 cc. of saturated Phenol Soln. Immediate appearance of bluish-white cloud signifies increased globulin. No advantage over the Ross-Jones test.
3. Glucose
 - (a) Qualitative test. (Hawk, p. 132)
 - (1) Five-tenths cc. spinal fluid are added to 5 cc. of Fehling's solution (diluted 1-5 and boiled in a water bath for 5 minutes. Results are reported as slight, moderate, or heavy.
 - (b) Quantitative test. (Hawk, p. 287)
 - (1) A modification of Benedict's picric acid method for sugar is used.
4. Lange's colloidal gold. (Ref.: Warwick and Nixon, Arch. Int. Med., Feb. 1920, Vol. 25)
 - (a) Technic
 - (1) Ten clean test tubes are set in a rack. To the 1st tube add 0.8 cc. of 0.4% NaCl soln., and to the remaining tubes add only 1 cc. of the salt soln. To an eleventh tube use only 1 cc. of the same salt soln. and 5 cc. of the gold soln. as a control.
 - (2) To the 1st tube add 0.2 cc. spinal fluid. Draw mixture back and forth with a 1 cc. pipette, graduated in tenths. Transfer 1 cc. to the next tube and so on through the last tube, discarding the last cc. Dilutions begin at $\frac{1}{10}$ and end at $\frac{1}{5120}$.
 - (3) Add to each tube 5 cc. of the gold soln.
 - (4) Shake each tube well.

(5) 0.4% NaCl soln. may be kept as a stock solution.

(b) Reaction

(1) Positive reaction begins at once and intensifies with complete precipitation in 8 to 12 hours.

(2) Syphilitic appears before the paretic curve.

(3) Lange's Colloidal Gold Numbers—

unchanged	0	deep blue	3
bluish red	1	gray blue	4
reddishblue	2	colorless	5

(4) Classification of the numbers.

(a) Zone 1 is 1/10 to 1/160, called "paretic," as 5555543000.

(b) Zone 2 is 1/40 to 1/160, called "syphilitic," as 0023311000. The maximum color is usually III, seldom V in the 4th or 5th tube. The first one or two tubes are unchanged.

(c) Zone III is 1/160 to the last tubes, called "meningitic". The curve is in the right half of the set, maximum in 7th or 8th tube, as 0000013310.

(c) Controls

Fluids of known reaction obtained from Ionia State Hospital for Criminal Insane.

(d) Clinical Interpretation

(1) Cell count:

(a) Five and less are called negative.

(b) More than 5 called positive.

(c) Plus-minus refers to reactions that are slightly or faintly positive, and if accompanied by other negative findings are of no significance; especially true of the Nonne test.

(2) Gold curve:

(a) Paretic or dementia paralytic reactions are marked. Rarely negative in untreated cases. Commonly gives reduction in Zone I and II. Persists even when patient is receiving intensive treatment.

(b) Syphilitic, tabes dorsalis, or lues have well marked curves in early cases.

(c) Cerebrospinal syphilis has striking value. 85% give some reduction, and 75% definite curves. 18% gold curve gave only an indication of pathologic conditions.

- (d) Multiple sclerosis has curve more frequently in Zone II and an average cell count of 14. (1.6 in normal fluids)
- (e) Brain and spinal cord tumors give a curve in Zone III and unaccompanied by meningitic symptoms is of definite diagnostic value and of special value when a negative or faintly positive Nonne and a low cell count.
- (f) Meningitis gives a curve in Zone III, a high cell count, 2 or 3 plus Nonne.

(e) Summary:

- (1) Colloidal gold test most delicate of routine spinal-fluid reactions.
- (2) Does not replace any other test, but of independent value.
- (3) Importance in early diagnosis of neurosyphilis.
- (4) Various curves are not specific, but of diagnostic value with other clinical findings and laboratory findings.
- (5) Does not parallel clinical signs or give definite evidence of improvement under treatment.
- (6) Patients with no involvement of the central nervous system or who are nonsyphilitic give no colloidal gold curve.
- (7) Clear cut cases of tabes dorsalis may show all spinal fluid reactions negative both before and after treatment.
- (8) A curve in Zone III with negative cell count and negative or faintly positive globulin is strongly suggestive of brain or cord tumor or myelitis.
- (9) Curves in Zone II and I may be found in multiple sclerosis and brain abscess.
- (10) All cell counts above 5 are pathogenic but of no value in indicating the duration or severity of the process or the improvement.
- (11) The reaction should be included in every spinal fluid analysis and every neurologic examination as well as examination in general syphilis.

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- (2) The Colloidal Gold Reaction.—Weston. Am. J. Syph., 3:266, 1919.
- (3) Cerebrospinal Fluid and the Colloidal Gold Test.—Felton. N.Y. M. J., 105:1170, 1917.
- (4) Interpretation of Paretic Curve in Lange's Colloidal Gold Test.—Loyd, James & Thompson. Arch. of Neurol. & Psych., Feb., 1921.

III. Mother's Milk

1. Procedure

(a) Specific gravity obtained with a urinometer

(b) Lactose

(1) The sugar titration method of Folin and McEllroy as modified by Folin and Denis for application to milk is used. (See p. 139.)

(c) Fat

(1) Babcock's method used.

Five cc. of milk are placed in a small Babcock tube graduated for reading fat up to 5%. Sufficient commercial sulphuric acid is now added to fill the body of the tube and the tube rotated for several minutes to secure a homogeneous mixture of acid and milk. The neck of the tube is then filled with a 1 to 1 mixture of concentrated hydrochloric acid and amyl alcohol. The tube is then centrifuged for 2 minutes and the fat read off by means of the scale on the neck.

(d) Protein. (Hawk, p. 345)

(1) The total nitrogen is determined on 5 cc. of milk by the Kjeldahl method and the protein obtained by multiplying this figure by the factor 6.37.

IV. Stomach Contents. (Hawk, pp. 172-77)

1. Microscopic examination

2. Titrated for

(a) Total acidity

(b) Free acidity

(c) Free hydrochloric acid

3. Test for blood

(a) Benzidine or ortho tolidin test used

4. Lactic acid

(a) Kelling's Ferric Chloride test used

(1) If positive confirm by Strauss' Ether Ferric Chloride test.

V. Feces. (Hawk, p. 236)

1. For occult blood use Benzidine or ortho tolidine test

VI. Urine.

1. Routine

(a) Specific gravity taken with urinometer

(b) Sediment examination

(1) 15 cc. of urine are centrifuged and the sediment examined under the microscope.

(c) Glucose

- (1) Tests for sugar are made with Benedict's solution.

(d) Albumin. (Ref.: Hawk, p. 452 and 454)

- (1) The heat coagulation test for albumin is used whenever possible. In old urines with heavy contamination of bacteria which cannot be obtained clear, Heller's ring test using *Robert's reagent* is used. (Hawk, p. 440)

(e) Indican

- (1) Indican is run on all urines except those received from life insurance companies and large surveys from the Industrial School. For this test *Obermeyer's reagent* is used. (Hawk, p. 639)

(f) Blood

- (1) The ortho tolidine reagent is used. The test is made only on suspicious looking specimens.

(g) Acetone bodies

- (1) Acetone bodies are tested for by Legal's sodium nitroprusside test and Gerhardt's ferric chloride test. (Hawk, p. 452)

2. Quantitative determinations in urine

(a) Glucose

- (1) Quantitative test for sugar is made by the method of Folin and McEllroy, which is the same as that described above for the titration of sugar in milk.

(b) Albumin

- (1) Purdy's centrifuge method is used. (Webster, p. 288)

Ten cc. of clear urine is placed in a 15 cc. graduated centrifuge tube. To this is added 3 cc. of 10% potassium ferrocyanide and 2 cc. of 50% acetic acid. The solutions are mixed and the tube allowed to stand 5 minutes. It is then centrifuged 3 minutes at 1500 revolutions and the volume of the precipitate read. Each $1/10$ cc. represents 1% of precipitate and this is multiplied by .21 to give the per cent by weight of albumin.

(c) Chlorides. (Ref.: Folin, p. 169. Hawk, p. 572)

- (1) The chlorides are precipitated with standard silver nitrate and the excess titrated back with standard ammonium sulphocyanate using ferric ammonium sulphate as an indicator.

- (d) Phosphates. (Ref.: Folin, p. 163. Hawk, p. 568)
 - (1) The phosphates are titrated hot with standard uranium acetate solution, using powder potassium ferrocyanide on a spot plate as an outside indicator.
- (e) Titratable acidity. (Ref.: Folin, p. 165. Hawk, p. 499)
 - (1) Twenty cc. urine is titrated with N/10 sodium hydroxide in the presence of potassium oxalate, using phenol phthalin as the indicator.
- (f) Total Nitrogen. (Ref.: Folin, p. 59. Hawk, p. 522)
 - (1) The total nitrogen is determined on 5 cc. of urine by the Kjeldahl method.
- (g) Urea. (Ref.: Hawk, p. 514. Folin, p. 135)
 - (1) The urea is determined on .5 cc. of urine by the Urease method, the ammonium found being aerated into standard hydrochloric acid.
- (h) Ammonia. (Ref.: Hawk, p. 519. Folin, p. 123)
 - (1) The ammonia in 5 cc. of urine is set free by the addition of saturated potassium carbonate and the ammonia is then carried over by an air current into a flask containing a measured amount of $\frac{N}{50}$ hydrochloric acid.
- (i) Creatinine. (Ref.: Folin, p. 147. Hawk, p. 526)
 - (1) The color obtained when creatinine is allowed to react with a saturated picric acid solution in the presence of alkali is the basis of the colorimeter estimation of creatinine.
- (j) Creatin. (Ref.: Folin, p. 149. Hawk, p. 529)
 - (1) Creatin may be obtained by first changing it to creatinine by boiling it with acid and then carrying out the above process. The increase in the value obtained being due to creatin.
- (k) Uric Acid. (Ref.: Folin, p. 145)
 - (1) Uric acid is determined colorimetrically by Benedict's new method. This is an improvement in Folin's longer method.
- (l) Acetone bodies. (Ref.: Hawk, p. 552)
 - (1) The method is based on a combination of Shaffer's oxidation of B hydroxybutyric acid to acetone and Denige's precipitation of acetone as a basic mercuric sulphate compound which is filtered off and weighed.

REFERENCES

Hawk's Practical Physiological Chemistry, 7th Edition, 1921; Folin's Laboratory Manual, 3rd Edition, 1922; and Webster's Diagnostic Methods, 7th Edition, 1920.

Colloidal Gold Solution

I. Technic for making gold solution

1. Double distilled spring water—1000 mls.
2. Heat water slowly in 2-liter flask to 50° C.
3. Heat rapidly to 60° C., and add 10 mls. of 1% gold chloride and 7 mls. of a 2% solution of potassium carbonate (Merck).
4. At 80° C. add 10 drops of 1% solution of oxalic acid.
5. At 90° C. the flask is removed from the flame, and slowly, drop by drop, 5 mls. of 1% solution of liquor formaldehyde is added.
6. The flask is then shaken until a pink color appears, slowly changing to a violet, then to a deep red with a golden shimmer. The solution is clear to transmitted light.
7. Solution must remain unchanged when set up with a normal fluid.
8. It must give a typical curve when set up with a paretic fluid.
9. Five mls. must be precipitated completely in 1 hr. by 1.7 mls. of 1% salt solution.
10. It must be neutral to a 1% solution of alizarin red in 50% alcohol.
11. To test the neutrality, 5 cc. of gold solution is put in a test tube with 2 or 3 drops of alizarin indicator added.
12. To adjust the solution to neutrality, use ten clean test tubes. To each add 1 cc. of distilled spring water. To the first tube add 1 cc. N/50 HCl or NaOH. From 1st tube transfer 1 cc. of the mixture to the second tube and so on through the last, discarding the last 1 cc. Dilutions are as follows: 0.5 cc. HCl, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078125, 0.00390625, 0.001953125, 0.000976. To each tube add 2 or 3 drops of Alizarin red, and 5 cc. of the gold solution. Color changes from canary yellow in acid to brownish red in neutral, deeper dark red in faintly alkaline, to deep purple in strongly alkaline solutions. Select the neutral tube. The amount of acid or alkaline in that tube is divided by 5 to determine the amount of acid or alkaline to neutralize 1 cc. of gold solution. This number is multiplied by the number of cc. to be corrected, and the resulting amount of N/50 acid or alkaline is added to the solution which is then ready for use. A deep dark red with a purplish tinge in the upper layers as well as the deep purple is a definite sign of an alkaline solution. No red without the brownish tinge should be accepted.
13. A satisfactory solution is very stable, kept corked in a dark cupboard, will keep for months. A fine black precipitate, or even surface mold which occurs in hot weather, will not interfere with the reaction. Neutral solutions are of vital importance.

Determination of Lactose in Milk

Use a dilution of 1:4 (25 c. c. in 100 c. c. flask) for cow's milk and 1:5 (5 c. c. in a 25 c. c. flask) for mother's milk. In emergencies as when the quantity of milk available is small, a dilution of 1:6 (2 c. c. added to 10 c. c. of water) can be used.

The titration is made as follows: Into a large test tube introduce 2.8 to 3.4 of the diluted milk (that is, nearly the full amount expected), 5 c.c. of a 6% copper sulphate solution, a pebble to prevent lumping, and 4 to 5 gms. of a dry salt mixture (made by mixing in powdered form 100 gms. of disodium phosphate, 60 gms. of anhydrous sodium carbonate and 30 gms. of sodium or potassium sulphocyanate). Shake and boil gently for 4 minutes before adding any more milk. At the end of this time add more milk (0.02 c. c. to 0.10 c. c. depending on the amount of blue color remaining) and boil again. After each addition of milk, except the first, boil one minute. The total boiling period should be 5 to 7 minutes. For the measurement of the milk we recommend the 5 c. c. burette with capillary tip described in the paper on urine. Calculation: 4.04 times the degree of dilution 4, 5, or 6 and divided by the titration figure, gives the per cent of lactose present.

REFERENCE:

Folin and Denis, Jr. *Biol. Chem.*, 33: 52, 1918.

A Simple Technique for the Determination of Calcium and Magnesium in Small Amounts of Serum

Method:

1 or 2 c.c. of serum are measured into an ordinary 15 c. c. graduated centrifuge tube containing 2 c. c. of water. 1 c. c. of a saturated solution of ammonium oxalate is then added and the sample mixed. The mixture is allowed to stand for half an hour. The volume is then made up to 6 c. c. with distilled water and the sample again mixed. The tube is centrifuged at 1300 revolutions per minute for 10 minutes. All but 0.3 c. c. of the supernatant fluid is syphoned off. The precipitate is mixed with the remaining fluid and 2 per cent ammonia (2 c. c. of concentrated ammonia to 98 c. c. of water) is added up to 4 c. c. and mixed. The tube is then centrifuged for 5 minutes. All but 0.3 c. c. of the supernatant fluid is again syphoned off. The last step is repeated twice, making three washings in all. The tube is centrifuged for 5 minutes after each washing. After the last washing the supernatant fluid is syphoned off. The crystals are suspended in the residual liquid and dissolved in 2' c. c. of approximately N sulfuric acid, heated in the water bath for a few minutes, and then titrated to a definite pink color, which persists for at least 1 minute, with a 0.01 N potassium permanganate. This is delivered from a microburette graduated in 0.02 c. c.

Calculations:

The number of c.c. of 0.01 N potassium permanganate used minus the blank (volume of 0.01 potassium permanganate required to produce the same intensity of color in an equal volume of water, usually .04 c.c.) $\times 0.2 =$ the number of mg. of calcium in the sample.

Reagents

The only reagent that must be quantitatively accurate is the 0.01 N sodium oxalate (Sorensen). This is used to standardize the permanganate. An 0.1 N sodium oxalate is prepared in the usual way. Solution of the oxalate is facilitated by the addition of 5 c. c. of concentrated sulfuric acid. The solution is then diluted 10 times to make a 0.01 N sodium oxalate. The latter solution will remain unchanged for several months; the former indefinitely. The other reagents are prepared in the usual way.

REFERENCE:

Kramer and Tisdall, Jr. *Biol. Chem.* 47:475. (June) 1921.

Preparation of N/100 Potassium Permanganate

Dissolve 0.40 g. pure potassium permanganate crystals in one liter of redistilled water in a thoroughly clean Florence flask which has been rinsed with the same water. Digest at or near the boiling point for 36 hrs. A funnel covered with a watch-glass may be used as a reflux condenser. Cool and allow to stand over night. Without disturbing the sediment of manganese oxides, filter with gentle suction through a 3-in. Buchner funnel lined with ignited asbestos. Both funnel and filter flask should be rinsed with redistilled water. Transfer the permanganate solution to a glass stoppered bottle free from traces of organic matter. The solution should be kept in the dark when not in use. If the asbestos becomes clogged with oxides these may be dissolved out with hot concentrated hydrochloric acid, followed by washing with redistilled water without disturbance of the pad.

After standing two or three days this permanganate solution may be conveniently standardized against N/50 oxalic acid (0.1261 g. pure crystals to 100 c. c.) or sodium oxalate of similar strength. To 10 c. c. of the oxalic acid solution add 10 c. c. of 10 per cent sulfuric acid which has been treated with just sufficient permanganate solution to give it a faint pink color. Place in a water bath at 65° C. for a few minutes. Then titrate at once to a definite pink color which persists for at least a minute. Correct for the blank obtained by titrating 10 c. c. of the sulfuric acid and the same volume of water to the same end-point.

If kept in a dark place, the oxalic acid solution used in standardization does not lose appreciably in strength in from ten days to two weeks. Ordinarily the permanganate solutions after they have stood several days will not vary over 0.1 per cent per week (see Table I). On account of the sensi-

tivity of the reagent it is, nevertheless, desirable to check it up rather frequently. This also serves as a control on technique.

REFERENCE:

Halverson and Bergeim, Jr. *Ind. & Eng. Chem.* 10:120. 1918.

Gum Mastic Test

Reagents

Ten grams of commercial gum mastic are dissolved in 100 c. c. of absolute alcohol, and the resultant cloudy fluid is filtered several times until a clear straw colored solution is obtained. This stock solution is kept in glass stoppered bottles at room temperature and does not deteriorate on standing. The emulsion is prepared with 1 c. c. of the stock solution added to 9 c. c. of absolute alcohol, which is then added, with gentle mixing, to 40 c. c. of once distilled water.

Procedure

In setting up the test, ten small tubes are employed. To the first tube in the series are added 1.5 c. c. of a stock salt solution (99 c. c. of 1.25 per cent sodium chloride solution plus 1 c. c. of 0.5 per cent potassium carbonate solution), and to each of the remaining tubes 1 c. c. is added. In the first tube, there is placed 0.5 c. c. of the spinal fluid to be tested. After mixing this dilution of spinal fluid, a titration is made throughout the series by transferring 1 c. c. from the first to the second tube, from the second to the third, etc., 1 c. c. being finally discarded from the last tube. Finally, 1 c. c. of the mastic emulsion is added to each tube. After mixing, the tubes are allowed to stand over night at room temperature, and the results are read in the morning. No special precautions regarding absolute cleanliness of glassware, such as are necessary for the gold test, need be observed.

The standards employed for reading results and typical readings of various types of abnormal fluids are shown in Table 1.

TABLE 1.—SHOWS THE STANDARDS EMPLOYED IN READING THE COLLOIDAL MASTIC TEST, WITH EXAMPLES OF TYPICAL CURVES.
EXAMPLES OF COLLOIDAL TESTS.

	Tubes.									
	1	2	3	4	5	6	7	8	9	10
Dilutions of spinal fluid.....	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048
Paretic zone.....	5	5	5	5	5	4	3	2	1	0
Syphilitic zone.....	0	1	1	2	3	3	1	0	0	0
Mastic "3".....	3	3	2	1	0	0	0	0	0	0
Negative.....	2	2	1	0	0	0	0	0	0	0

Hematology

I. Cytology. Ref.: Stitt, p. 292.

1. Obtaining blood:

Puncture ear or finger by quick stab of needle until blood flows easily in large drops. Always wipe off skin and obtain fresh drop for each test.

2. Red blood cell count:

To determine number of red blood cells in c.mm., draw blood into red blood cell counting pipette up to the 0.5 mark. This must be exact and there must be no bubbles in the column of blood. Now draw Hayem's diluting fluid into pipette exactly to the mark 101, making a dilution of 1 in 200. Mix thoroughly at least one minute, expel fluid below bulb. Place drop of diluted blood (1:200) in Thoma-Levy counting chamber, Neubauer ruling. To do this, first place cover glass over chamber and touch edge of chamber, allowing diluted blood to flow under cover glass until chamber is just filled. Allow cells to settle for a few minutes and then observe under 16 mm. objective. If cells are uniformly distributed, proceed with count as follows: Count 80 of the small squares; for example, count four triple ruled rectangles, each containing 20 of the small squares. These 80 small squares are $1/50$ of the total number of 4000 in one c. mm. So the number of red cells counted in the 80 small squares must be multiplied by 50 and then by the dilution of the cells which is 200. In other words, multiply the number of red cells counted in 80 of the smallest squares by 10,000 or add 4 ciphers. Repeat above procedure and report average of the two counts.

3. White blood cell count:

To determine the number of white blood cells in c. mm., draw blood into white blood cell counting pipette up to the 0.5 mark. This must be exact and there must be no bubbles in the column of blood. Now draw diluting fluid (1.0% acetic acid) into pipette exactly to mark 11, making a dilution of 1 in 20. Mix thoroughly for at least one minute. Expel fluid below bulb. Place drop of diluted blood in Thoma-Levy counting chamber, Neubauer ruling. To do this, first place cover glass over chamber and touch edge of chamber with tip of pipette, allowing diluted blood to flow under cover glass until chamber is just filled. Allow cells to settle for a few minutes and then observe under 16 mm. objective. If cells are uniformly distributed, proceed with count as follows: Obtain the average number of white cells in a 1 mm. square after counting

the 1 mm. squares at four corners of field. As the depth of the chamber is $1/10$ mm., multiply the number of cells in a 1 mm. square by 10. Then multiply by the dilution of blood, 20. In other words, multiply the number of white cells per 1 mm. square by 200, and it represents the number of white cells per c. mm. Repeat above procedure and report average of the two counts.

4. Differential blood count:

Use absolutely clean glass slides. Touch slide to small fresh drop of blood and lay slide on table. Draw second clean slide back into blood and push across to other side, the blood following the second slide. The thickness of smear can be regulated by varying the angle between the two slides. Air dry before staining. Make several smears from each case.

Stain smear by Wright's stain. Leave undiluted stain on smear for one minute. Dilute with an equal quantity of distilled water and allow to remain for five minutes. Wash thoroughly with distilled water, dry and examine for distribution of white cells with 16 mm. objective. If the distribution is uniform, make count under oil immersion. Use mechanical stage and use definite scheme of covering field so as to avoid same fields twice. Count 200 white cells and record the percentage of polymorphonuclear neutrophiles, basophiles and eosinophiles; large and small lymphocytes; large mononuclears and transitionals. Make a careful examination of the red cells in typical fields to determine whether they present a normal appearance. Record presence of poikilocytes; macrocytes and microcytes; achromatic or polychromatophilic erythrocytes. Give the number of megaloblasts, normoblasts and microblasts found in fields examined. Look also for punctate basophilia of red cells. Ref.: Hematological Atlas.

II. Examination of blood for malarial parasites. Ref.: Wood's Chemical and Microscopic Diagnosis, p. 191.

1. Make smear of blood as for differential count. Stain by Wright's method. Examine with oil immersion for presence of malarial parasites within red cells.

III. Blood for compatibility test.

1. Two c. c. citrated blood from recipient and from donor. Centrifuge blood and pipette off serums. Wash red cells twice with sterile saline. Make up a 1:10 suspension red cells from recipient and donor (0.2 c. c. packed cells + 0.8 c. c. saline). Set up test as follows:

	1	2	3
Row 1 Donor's Cells	0.05 cc. 1:10 susp. D. cells + 0.2 cc. recipient's ser. + 1.0 cc. saline	0.05 cc. 1:10 susp. D. cells + 0.2 cc. donor's ser. + 1.0 cc. saline	0.05 cc. 1:10 susp. D. cells + 0.2 cc. saline + 1.0 cc. saline
Row 2 Recipient's Cells	0.05 cc. 1:10 susp. R. cells + 0.2 cc. donor's ser. + 1.0 cc. saline	0.05 cc. 1:10 susp. R. cells + 0.2 cc. recipient's ser. + 1.0 cc. saline	0.05 cc. 1:10 susp. R. cells + 0.2 cc. saline + 1.0 cc. saline

Observe immediately for agglutination. Incubate at $37\frac{1}{2}^{\circ}$ one hour and observe again for agglutination and also for hemolysis.

IV. Blood grouping (microscopic method). (Jansky's Classification).

1. To test unknown corpuscular suspension having on hand sera of Group II and Group III.

Cell Suspension. Collect one large drop of blood from a prick in finger or ear in 1 c. c. of 1.5% sodium citrate solution in 0.9% saline or make up 1:10 saline suspension of washed red cells.

Test. One drop cell suspension + 1 drop Group II serum.
One drop cell suspension + 1 drop Group III serum.
One drop cell suspension + 1 drop saline.

Interpretation.

Agglutination of unknown cells with Group II serum indicates Group III.

Agglutination of unknown cells with Group III serum indicates Group II.

Agglutination of unknown cells with both II and III serum indicates Group IV cells.

No agglutination of unknown cells with either II or III indicates Group I cells.

2. To test unknown serum having on hand corpuscular suspension of Group II and Group III.

Serum. Collect about 2 cc. blood from vein in arm. Allow to clot and remove clear serum for test.

Test. One drop unknown serum + one drop cell suspension Group II.

One drop unknown serum + one drop cell suspension Group III.

Controls:

One drop saline + one drop cell suspension Group II.

One drop saline + one drop cell suspension Group III.

Interpretation.

Agglutination of Group II corpuscles by unknown serum indicates Group III serum.

Agglutination of Group III corpuscles by unknown serum indicates Group II serum.

Agglutination of both Group II and III corpuscles by unknown serum indicates Group I serum.

Agglutination of neither Group II nor Group III corpuscles by unknown serum indicates Group IV serum.

REFERENCES:

Stitt's Practical Bacteriology and Blood Work, 7th Edition, 1923.

APPENDIX

APPENDIX

- A—Medical Supply Depot.
- B—The Distribution of Biological Products.
- C—Extension Work.
- D—Special Laboratory Accessories.
- E—Instrument Shop.
- F—Laboratory Blanks.

A--MEDICAL SUPPLY DEPOT

The technical supervision of the Medical Supply Depot is one of the auxilliary functions of the director of laboratories. This depot is located on the mezzanine floor of the New State Building directly under the laboratory. Its functions are the purchasing and furnishing of medical, surgical, hospital and laboratory equipment and supplies to all state institutions except the University of Michigan and the Michigan State College.

Central Michigan Normal School.....	Mt. Pleasant
Ionia State Hospital.....	Ionia
Industrial School for Boys.....	Lansing
Kalamazoo State Hospital.....	Kalamazoo
Maekinae State Park Commission.....	Mackinae Isle
Michigan College of Mines.....	Houghton
Michigan Employment Institution for the Blind.....	Saginaw
Michigan Farm Colony for Epileptics.....	Wahjamega
Michigan Reformatory.....	Ionia
Michigan Home and Training School.....	Lapeer
Michigan School for the Blind.....	Lansing
Michigan School for the Deaf.....	Flint
Michigan State Prison.....	Jackson
Michigan State Normal College.....	Ypsilanti
Michigan Soldiers' Home.....	Grand Rapids
Michigan State Public School.....	Coldwater
Northern State Normal College.....	Marquette
Newberry State Hospital.....	Newberry
Pontiac State Hospital.....	Pontiac
State House Correction & Branch Prison.....	Marquette
State Psychopathic Hospital.....	Ann Arbor
State Industrial School for Girls.....	Adrian
State Sanatorium.....	Howell
Roosevelt American Legion Hospital.....	Camp Custer
Traverse City State Hospital.....	Traverse City
Western State Normal School.....	Kalamazoo

The idea of the Medical Supply Depot originated in the mind of the Commissioner of Health, following a memorandum* sent to him in October, 1921. The increased

*Memorandum to: Dr. R. M. Olin. From: Dr. C. C. Young. October 3, 1921.

The following table will give you a comparison between jobber's prices and factory prices:

Items.	Jobber's Price.	Factory Price.	Quantity Lots.	Estimate of Annual Use.
Glass slides, 3 x 1.....	\$1.65 per gr.	\$0.87 per gr.	20,000	50,000
Test Tubes, 3 x 3/8.....	3.00 per gr.	1.05 per gr.	50,000	100,000
Test tubes, 3 1/2 x 1/2.....	3.10 per gr.	1.50 per gr.	50,000	100,000
Test tubes, 5 x 3/8.....	3.50 per gr.	1.75 per gr.	10,000	15,000
Shell vials, 2 dram.....	1.40 per gr.	.60 per gr.	50,000	100,000
Cover glasses.....	2.00 per oz.	.90 per oz.	100 oz.	50 oz.
Aluminum wire, 14 gauge.....	2.50 per lb.	.413 per lb.	100 lb.	200 lb.
Filter paper.....	16.50 per ream	8.20 per ream	10 reams	20 reams
Cotton.....	40 per lb.	.19 per lb.	500 lbs.	500 lbs.
Sputum jars.....	30.00 per M	13.00 per M	144,000	25,000
Petri dishes.....	.26 each	.20 each	5,000	2,500
Pipettes.....	50.00 per gr.	16.00 per gr.	3 gross	20 gross
Syringes, 5 cc.....	35.00	13.00	1 doz.	5 doz.

demands upon the laboratory made it impossible for it to operate within the limited appropriation available, as long as supplies were purchased as needed from jobbers. By October, 1921, we had some estimate of the minimum quantities of material that would be used for a two-year period. The manufacturers of the various laboratory supplies were consulted and it was found that a fifty per cent net saving could be effected if we could enter into an agreement with the factory to furnish us all the material we used and place a definite order for wholesale lots.

Doctor Olin, in turn, took up the matter with the State Administrative Board which authorized sufficient funds for the purchase of standard glassware and supplies, in such a manner as to get wholesale factory prices. It will be noted that these prices show a saving in some instances as great as 300 per cent; in one instance, that of aluminum wire which is used in the manufacture of swab wires for throat cultures, shows a saving of over 600 per cent. This method of buying cut our operating cost from \$22,000 in 1920 to \$15,000 in 1922. The merit of buying small items in quantities sufficient to get wholesale prices was obvious.

(Compared to the expenditures of the State of Michigan for food and clothing and other supplies for the institutions these expenditures are, of course, incidental.)

As a result the State Administrative Board appropriated a revolving fund of \$60,000 for the purchase of medical, surgical, hospital and laboratory supplies, which were to be kept in a store under the supervision of the Commissioner of Health and shipped out on requisition to the various state institutions.

This store has been in operation about eighteen months and is one of the greatest conveniences of the laboratory, in that we have an ample supply of chemicals, glassware and other supplies on hand at all times, sufficient to meet any emergency and purchased at the lowest market price. We are able to take advantage of seasonal low prices and special factory clearance sales. As an illustration of this point, there was a stock of several thousand bandages in one of the large surgical supply houses which had been rejected by the Government on account of being a few inches short of the specified yardage required on Army purchases. These were bought for twenty cents a pound, the market price being \$1.40. In the summer of 1923, there was every indication that cotton would go up in the fall, so purchase was made of 200,000 yards of gauze and ten tons of absorbent cotton, which is sufficient to supply the institutions for six or eight months. We are today selling gauze to our institutions at twenty per cent under the wholesale market, and cotton at thirty-two per cent under the wholesale market.

By entering into a price protection agreement with the pharmaceutical houses we are able to show a net saving of thirty-five per cent on their standard price formulae.

There are some difficulties of bookkeeping and emergency orders which have yet to be worked out to make this store a complete success, but on the whole it has not only justified its existence but has gradually acquired the unqualified support of the superintendents and purchasing agents of the institutions.

From the standpoint of the laboratory of the Michigan Department of Health, the Medical Supply Depot has become an almost indispensable institution, as the availability of material practically eliminates administrative details so often annoying in the operation of a diagnostic laboratory. The stock of material now represents approximately \$66,000 and is turned over twice a year. It is interesting as a matter of record that the entire residue of the Camp Custer Medical Supply Depot was purchased at seven per cent of its original cost to the Government and formed the nucleus of this stock. There was about \$50,000 worth of property which was dispensed to the state institutions at a total cost of about \$4,000.

B—THE DISTRIBUTION OF BIOLOGICAL PRODUCTS

Report of Biological Division January 1st, 1922, to January 1st, 1924

In accordance with Act 370 of the Public Acts of 1921, the free distribution of Diphtheria Antitoxin, Schick Test Material and Toxin-Antitoxin, for the treatment and prevention of diphtheria, went into effect January 1st, 1922. This Act was amended by the 1922-1923 legislature.

The contracts for the period from January 1st, 1922, to June 30th, 1923, were awarded as follows:

DIPHThERIA ANTITOXIN—H. K. Mulford Company
SCHICK TEST MATERIAL—The United States Standard Products Co.
TOXIN-ANTITOXIN—The United States Standard Products Co.

New contracts were let July 1st, 1923, covering a one-year period as follows:

DIPHThERIA ANTITOXIN—The Gilliland Laboratories
SCHICK TEST MATERIAL—H. K. Mulford Company
TOXIN-ANTITOXIN—E. R. Squibb & Sons

On the first contracts the following amounts were purchased:

47,500 pkgs. 1000 unit antitoxin at.....	.22	\$10,450.00
15,500 pkgs. 5000 unit antitoxin at.....	.545	8,447.50
17,067 pkgs. 10000 unit antitoxin at.....	.99	16,896.33
11,250 pkgs. 20000 unit antitoxin at.....	1.95	21,937.50
140,000 c. c. of Toxin-Antitoxin.....	.04	5,600.00
1,000 pkgs. Schick Test Material.....	.15	150.00
		\$63,481.33

On the second contracts the following amounts were purchased from July 1st, 1923, to January 1st, 1924:

25,000 pkgs. 1000 unit antitoxin at.....	.095	\$2,375.00
6,500 pkgs. 5000 unit antitoxin at.....	.285	1,852.50
8,000 pkgs. 10000 unit antitoxin at.....	.475	3,800.00
8,000 pkgs. 20000 unit antitoxin at.....	.855	6,840.00
90,000 c. c. of Toxin-Antitoxin.....	.022	1,980.00
1,008 pkgs. Schick Test Material.....	.14	141.12
		\$16,988.62

Before this law went into effect antitoxin was purchased for individual cases in rural communities. In some cities it was distributed free to indigents only. In Detroit and Grand Rapids it was distributed free, the average price paid being \$.75 per 1000 units. For the two-year period this would have cost the State, under the old method, \$613,627.50. Our total cost was \$72,598.83, making a net saving to the State of Michigan of \$541,028.67, which is more than the cost of operating the whole Health Department of the State of Michigan for the period of two years.

Four hundred and forty-four distributing depots have been established, which places the antitoxin within ten miles of every physician in Michigan. The distribution is handled by druggists, except in the larger cities where it is taken care of by the City Health Department.

The original amount of antitoxin stocked with the distributors was determined by the population of the town and the number of cases of diphtheria reported from that territory during the preceding two years.

The method of keeping records has been made as simple, yet as thorough as possible. When shipments are made shipping orders are made in duplicate for the required amount. The shipping clerk records the serial numbers, lot numbers and expiration dates of each package on the back of the duplicate copy. The original copy is enclosed in the package and is returned to us by the distributor upon receipt of the package checked and signed by him, thus letting us know that the shipment has been received. The duplicate copy is filed after we have posted the information on the distributor's card. These cards have space for both shipments and for distribution. Shipments are posted in detail showing the date of shipment, amount, size, serial numbers, lot numbers and expiration dates. The distributor is supplied with books of requisition blanks and when he dispenses the antitoxin he makes the report on these blanks, which are in triplicate. They cover the following information: date, quantity, size, serial numbers, physician's name, patient's name, age and address. They are signed by both the distributor and the physician. The original and duplicate copies are mailed to us, the triplicate retained by the distributor for his files. Upon receipt of our copies we immediately post the information, in detail, on the distributor's card. The duplicate copy is sent to the Communicable Disease Bureau to be checked against cases reported. When the distributor's card shows that 50% of his stock has been dispensed we immediately replace it, thus keeping him supplied and making it unnecessary for him to order except in cases of emergency, when he may telegraph or telephone at our expense.

A safety test is made on every lot of each product before it is distributed and samples reserved for any future tests that may be necessary.

Our records of Stock on Hand are kept as carefully as the distributor's accounts. Antitoxin purchased and shipped is recorded daily on stock cards for this purpose, enabling us to know the number of packages of each size on hand each day.

The distribution of Schiek Test Material and Toxin-Antitoxin is done direct from this department owing to the necessity of keeping these products in the most careful storage. These are dispensed to physicians only. Shipments are recorded on cards for this purpose but the physician is not required to report when using it. Stock cards are kept in the same manner as those covering antitoxin.

The free distribution of Typhoid Vaccine went into effect July 1st, 1922. This product is made in our own laboratory at a cost of \$.04 per c. c. We have distributed 26,000 c. c. of this.

The department also keeps a supply of the following biologicals which are sold to institutions and to physicians in cases of emergency. These are billed at cost plus 10%:

- Smallpox Vaccine
- Tuberculin Old for Von Pirquet Test
- Botulinus Antitoxin
- Tetanus Antitoxin
- Influenza Bacterin
- Gonococcic Serobacterin
- Acne Serobacterin
- Ragweed Pollen Extract (Hay Fever)
- Antistreptococcic Serum
- Antipneumococcic Serum
- Antimeningitis Serum
- Neo-Arsphenamine

C—EXTENSION WORK

The extension work of the laboratory service has two purposes, one, that of broadening the service of diagnostic aid to practicing physicians, and the other, to tone up the viewpoint of the laboratory workers. It is a recognized fact that in organizations away from academic centers where the work of the personnel is focused primarily on routine procedures, they have the tendency to get into a rut. We have attempted by means of our extension work to keep the group interested in the ultimate application of their laboratory work and to avoid as much as possible the industrial production attitude toward large numbers of specimens.

Training Laboratorians

Since the reorganization of the laboratory of the Michigan Department of Health, there have been six municipal laboratories started in the state for which we have trained the laboratorian. All of these municipal laboratories are now going concerns and are serving their community in such a way as to facilitate certain types of medical work. These individuals call upon the main laboratory for assistance when they get into difficulties and look to the Michigan Department of Health for many of their diagnostic reagents and much of their stock and special media, as well as relief for their vacation periods. We feel that this is conducive to a higher grade of work as the material is standardized and the results of the workers comparable.

Media Distribution

At the present time we are furnishing media to the following cities:

Adrian	Kalamazoo
Allegan	Lansing
Battle Creek	Mt. Pleasant
Bay City	Pontiac
Elmira	Port Huron
Flint	Saginaw
Harrisville	Muskegon
Ionia	Ludington
Jackson	Wahjamega

Media Prepared in Laboratory

LIQUID MEDIUMS.

Infusions..... \$1.00 per liter.

Beef, Veal, Liver and Beef Heart.

Broths:

Beef Extract.....	\$1.00 per liter.
Beef Infusion.....	1.00 " "
Beef Extract, sugar free.....	1.00 " "
Beef Infusion, salt free.....	1.00 " "
Beef Infusion, sugar free.....	1.00 " "
Toxin, for Virulence Tests.....	2.00 " "
Hydrogen Ion (Cooledge).....	1.00 " "
Beef Extract, single strength + Lactose.....	2.00 " "
Beef Extract, double strength + Lactose.....	2.00 " "
Aseptic Fluid Water.....	1.00 " "
Dunham's Peptone.....	1.00 " "
Beef Infusion + Dextrose.....	2.00 " "
Lactose Bile + Brilliant Green Solution.....	2.00 " "

Lactose Beef Extract + Gentian Violet.....	\$2.00	per liter.
Hilber's Brain Media.....	2.00	" "
Peptone Broth + Dextrose.....	2.00	" "
Peptone Broth for Methyl Red test.....	2.00	" "
Johnins Medium.....	2.00	" "
Hormone Bouillon.....	2.00	" "
Phosphate Dextrose, salt free.....	2.00	" "
Beef Infusion + Glycerine.....	1.00	" "
Muscle Sugar Free Broth Beef Extract + Carbohydrate....	2.00	" "
Dextrose, Salicin, Lactose, Inulin, Saccharose, Xylose, Maltose, Raffinose, Levulose, Arabinose, Inosit, or Dextrin.		
Muscle Sugar Free Broth Beef Extract + Carbohydrate....	10.00	" "
Dulcite or Adonite.		
Potato Extract.....	2.00	" "
Beef Extract Broth + Dextrose.....	2.00	" "
Milk.....	1.00	" "
Litmus and Brom Thymol Blue.		

SOLID MEDIUMS.

Stock Supplies.....	\$2.00	per liter.
2% Agar Beef Extract + Salt (Tube Medium)		
3% Agar Beef Extract + Salt (Plate Medium)		
1½% Agar Beef Extract, Salt Free (water analysis)		
1½% Agar Beef Infusion		
Agar Veal Infusion		
Agar Veal Infusion + Glycerine		
2% Agar Beef Infusion + Dextrose		
2½% Agar Beef Infusion + Glycerine		
Agar Beef Infusion + Gentian Violet Dye		
1½% Agar Beef Infusion + Starch		
Beef Heart Agar		
Iver Infusion Agar		
Gelatin		
Triple Sugar Andrade Agar		
Potato Extract Agar		
Semisolids:		
½% Agar Beef Extract, Muscle Sugar Free.....	\$2.00	per liter.
½% Agar Beef Infusion	2.00	" "
Muscle Sugar Free Agar + Carbohydrate.....	2.00	" "
Extrose, Lactose, Saccharose, Xylose, Salicin, Inosit, Inulin, Maltose, Riffinose, Arabinose, Dextrin, Levulose or Mannose.		
Muscle Sugar Free Agar + Carbohydrate.....	10.00	" "
Adonite or Dulcite		
Slants.....	\$0.04	per tube
Beef Heart Agar + 5% Blood		
Beef Infusion Starch Agar + 5% Blood		
Beef Infusion Starch Agar		
Double Sugar Andrade		
Beef Infusion Agar + Glycerine		
Beef Infusion Agar + Dextrose		
Potato		
Agar Beef Infusion + Serum		

Loeffler's serum medium
 Egg Medium
 Egg Medium + Gentian Violet Dye
 Blood Agar
 Coagulated Blood Agar
 Agar Beef Extract — $3\frac{1}{2} \times \frac{1}{2}$ and $5 \times \frac{5}{8}$ tubes
 Agar Beef Infusion
 Agar Beef Infusion Stabs
 Gelatin Stabs
 Triple Sugar Andrade
 Ascitic Fluid Infusion Agar
 Liver Infusion Agar

Plates.....\$.05 per plate + cost of petri dishes

5% Sheep's blood agar
 Coagulated Blood Agar
 Beef Extract Agar
 Beef Infusion Agar + Starch
 Beef Infusion Agar + Ascitic Fluid
 Endo's Medium (Typhoid-Dysentery Diagnosis)
 Endo's Medium (Water Analysis)
 Brilliant Green Agar
 Eosin Methylene Blue Agar
 Beef Infusion Agar + Gentian Violet
 Sodium Oleate

SOLUTIONS..... \$0.10 per oz.

Lactose Sugar 20% (Filtered Solution)
 Maltose Sugar 20% (Filtered Solution)
 Saccharose Sugar 20% (Filtered Solution)
 Lactose 20%-Saccharose 20% (Filtered Solution)
 Lactose 20%-Dextrose 2% (Filtered Solution)
 Sodium Oleate 2%
 Pakes
 Antiformin
 Ringers
 Potassium Permanganate $\frac{1}{2}\%$
 Sodium Bisulphite 5%
 Phenol 5%
 Physiological Salt

INDICATORS AND DYES..... \$0.25 per oz.

Basic Fuchsin 5%
 Brilliant Green .1%
 Aqueous Methylene Blue $\frac{1}{2}\%$
 Aqueous Eosin 3%
 Saturated Gentian Violet Solution
 Aqueous Gentian Violet Solution
 Andrade Indicator
 Brom Thymol Blue Indicator .4%
 Phenol Red .4%
 Litmus Indicator
 Phenolphthalein
 Methyl Red Alcoholic
 Sodium Suphite (not furnished in solution)

MEDALIA STANDARD FOR HYDROGEN-ION CONCENTRATION.... \$3.50 (Colorimeter Block included)

It is our expectancy to act as a source of supply for standard culture media, antigen diagnostic sera and standard cultures, and to continue the training of laboratory workers to fill vacancies whenever we are called upon.

Field Work

Members of the laboratory staff have been called out periodically to demonstrate the Schick test and active immunization against diphtheria. These demonstrations have been given before county medical societies, groups of doctors and nurses. During the year 1920 some 10,000 children were Schick tested and immunized. This work has gradually broadened until we feel that it is no longer a technical function of the laboratory but belongs to the administrative side of the Department of Health and it has now been turned over to the Bureau of Epidemiology. In 1919 and early in 1920 groups were sent out from the laboratory to make cultures of the throats of school children and report upon the carrier situation. As a result of this work approximately 100,000 children's throats were cultured in various communities. These carrier surveys, although they in themselves are not of very great public health importance in the control of diphtheria, had an enormous educational value. One community installed a municipal laboratory immediately after the visit of our workers. There is no doubt in the mind of the writer that the field work has been one of the contributory factors in the enormous growth of the laboratory service in Michigan.

Division of Instruction

After the Bureau of Laboratories had been in existence for about two years, requests began to come in for individuals to spend time in the laboratory observing the methods in use. These requests came from students who were taking courses in colleges and universities, from office workers of practicing physicians, and from physicians themselves, who wished to brush up their technic so that they could do some work in their own office. Up to September, 1923, we have had 12 volunteer workers who have spent on the average of thirty days each in the laboratory. In September, 1923, an agreement was entered into with the Department of Bacteriology of the Michigan Agricultural College to give the course in public health laboratory methods for that department. This fall we have had four students in the laboratory for the trimester. To meet the increase in this type of work we organized among the laboratory force the Division of Instruction, an outline of which follows with a general perspectus of the work to be accomplished. This was sent out to all the schools and colleges in the state. It is quite within the realm of possibility, that, as time goes on, these schools will take advantage of this Division of Instruction and send students here for practical training in routine public health laboratory methods. There is so much available material here that proficiency in the technology of the biological sciences can be acquired with much greater rapidity than it can in a college or university.

Bulletin

DIVISION OF INSTRUCTION

C. C. Young, Director

R. L. Kahn, Immunologist

M. Crooks, Bacteriologist

P. L. Kendrick, Assistant

P. C. Yull, Assistant

A. B. Haw, Physiological Chemist

L. D. Henry, Senior Bacteriologist

M. S. Marshall, Research Bacteriologist

A. Exworthy, Sanitary Bacteriologist

1. Observers. Any person having the necessary qualifications may visit the

laboratories for the purpose of observing methods. They are expected to conform to the Rules and Regulations of the laboratory.

2. Students in Applied Bacteriology for Nurses. A course in applied bacteriology is offered to women enrolled in nurses' training schools.

3. Students in Public Health Laboratory Procedure. A course in public health laboratory procedure is offered to students enrolled in a college or university.

4. Volunteer Workers. College graduates wishing experience in public health laboratory methods may volunteer for not less than three months' time in the laboratory. The work will be divided into routine laboratory work and confirmatory studies. Laboratory work will occupy a daily three-hour period. Each subject must be satisfactorily completed before taking up a new subject. Volunteer workers will conform to the Rules and Regulations of the laboratory, keep a daily record of all work done and make a weekly report to the Director of Laboratories. They shall not be responsible for routine diagnosis.

5. Lectures on Applied Medical Science. Weekly lectures will be given by specialists in their fields.

6. Journal Club. Thirty-seven scientific periodicals are reviewed by members of the laboratory staff. Articles on public health laboratory procedure are abstracted and submitted for publication to the American Journal of Public Health.

It is the feeling of the writer at the present time that this extension division of the laboratory is but a nucleus around which the probable development of the central laboratory will congregate in its ultimate development. The plans for the future are largely in that direction.

COURSE I. (Prerequisite: 1. General Bacteriology. 2. The Pathogenic Bacteria.)

This course is designed to give fundamental training in public health methods. Practice is offered in bacteriological diagnosis of diphtheria, tuberculosis, typhoid fever and gonorrhea. Instruction is given in the bacteriological examination of feces, urine, blood, spinal fluid, pleural fluid, water and milk. Serum diagnosis in syphilis is also covered.

"Pathogenic Microorganisms" by Park and Williams is recommended as a textbook.

COURSE II. Advance Work. (Prerequisite: Course I.)

COURSE III. Clinical Laboratory Procedure.—Haw.

Practice is offered in Blood Chemistry, Urinalysis, Hematology, Blood Grouping, Lange's Colloidal Gold Test.

Prerequisite: 1. Chemistry, Qualitative. 2. Lectures in Physiological Chemistry. 3. Histology.

COURSE IV. Research in Immunology.—Kahn.

Research work in Immunology is offered to qualified students.

COURSE V. Research in Bacteriology.—Marshall and Crooks.

The general research problems of the Division of Bacteriology offer special problems to qualified students.

COURSE VI. Public Hygiene.—Young.

A series of lectures on application of bacteriology to municipal hygiene, water supply, food supply, etc.

COURSE VII. Public Health Laboratory Administration.—Young.

Course I

A. Serum Diagnosis in Syphilis.—Kahn.

I. SUBJECT—THE WASSERMANN TEST.

1. Ingredients entering into test
 - (a) Patient's serum: Separation from clot, inactivation, etc.
 - (b) Antigen: Preparation and use in test
 - (c) Complement: Method of obtaining and using in test
 - (d) Amboceptor: Method of obtaining and using in test
 - (e) Sheep Cells: Method of obtaining and using in test
2. The test proper
Theoretical and practical considerations of the test as a whole.

II. SUBJECT—THE PRECIPITATION TEST

1. Ingredients entering into test
 - (a) Patient's serum
 - (b) Antigen
2. The test proper
Theoretical and practical considerations of the test as a whole.

B. Diagnostic Bacteriology:—Crooks.

I. SUBJECT—TYPHOID DIAGNOSIS. Bact. No. 1.

1. Preparation of Media.—Henry
2. Isolation and identification of organisms from
 - (a) feces
 - (b) blood
 - (c) urine
3. Agglutinations
 - (a) microscopic
 - (b) macroscopic

II. SUBJECT—DIPHTHERIA DIAGNOSIS. Bact. No. 1.

1. Preparation of Media.—Henry
2. Culture
3. Preparation and microscopic examination of stained preparations

III. SUBJECT—TUBERCULOSIS DIAGNOSIS. Bact. No. 2

1.
 - (a) microscopic examination of stained preparations
 - (b) animal inoculation
1. Pulmonary
 - (a) sputum
 - (b) pleural fluid
2. Of Genito-Urinary Tract
 - (a) urine
3. Meningitis
 - (a) spinal fluid

IV. SUBJECT—GONORRHEAL INFECTIONS.

1. Preparation of stains and staining methods
2. Microscopic examination of stained preparations

D—LABORATORY ACCESSORIES

At times, in our attempt to put laboratory service on an efficient basis, we have found it difficult to adapt articles that were in stock in the apparatus companies to the particular needs of our routine procedure. This has necessitated special designs and improvements of articles already in use. Some of the more important articles designed by the writer or by one of his associates following out his suggestions are included in this appendix.

Syringe for Schick Testing*

C. C. Young and Minna Crooks, Lansing, Mich.

In group Schick testing, speed and accuracy of dosage are essential. This syringe adds materially in giving an exact amount and increases the rapidity of administering diluted toxin for the Schick test.

An ordinary Luer 2 mil tuberculin syringe blank is graduated to deliver .2 mil between marks. Each .2 mil graduation mark completely encircles the barrel of the syringe. Thus no matter in what position the syringe comes to rest in the operator's hand after inserting the needle intradermally, the graduation for dosage is apparent.

This device will increase the number of injections per hour at least 20 per cent.

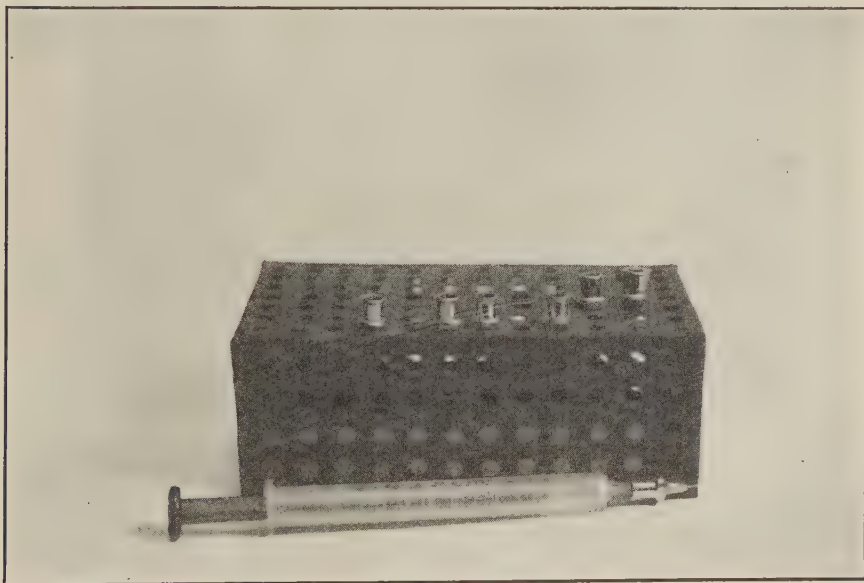


Figure 3.—Syringe for Schick Test.

A Rack for Hypodermic Needles

When working in groups where it is necessary to change needles after each inoculation, much time is wasted in fishing needles from sterilizer tray or other container.

We have found the little perforated table made of phosphor bronze (Fig. 3) a great time saver. It will hold perpendicularly any length needle from $\frac{3}{8}$ " to $1\frac{3}{4}$ ". The

* Reprinted from the Journal of the American Medical Association, 78:651, 1922.

dimensions $4\frac{3}{4}$ " x $2\frac{3}{4}$ " x 2" permit the use of two racks in any small electric sterilizers. There are 84 $\frac{3}{16}$ " holes arranged in seven rows of 12 each. The $\frac{3}{16}$ " diameter of the opening is just the proper size to receive and balance any standard slip joint hypodermic needle. The needles can be lifted without use of forceps by inserting with a rotary motion the receiving tip of the syringe into the needle sockets.

In giving toxin-antitoxin or typhoid vaccine, or in taking blood for the Wassermann test, this device will speed up the work. It has been repeatedly demonstrated that by using the rack instead of extracting needles from a tray with forceps, one can increase the number of bloods taken for Wassermann test 50 per cent.

The Wire Swab

To fit into the economic ideas of purchasing supplies, it was necessary to adapt the throat culture wire to the standard containers. Swab wire of various metals had been used and the most satisfactory one that had come to the attention of the writer was a small aluminum wire which was nicked at one end with a pair of scissors to hold the cotton swab. Swabs could be wrapped around the wire and the wire brought over the lip of the test tube as shown in Figure 4. The same size tube was utilized for this work that we had found most convenient for Loeffler's blood serum medium. It is a $3\frac{1}{2}$ x $\frac{1}{2}$ inch tube illustrated in Figure 7.

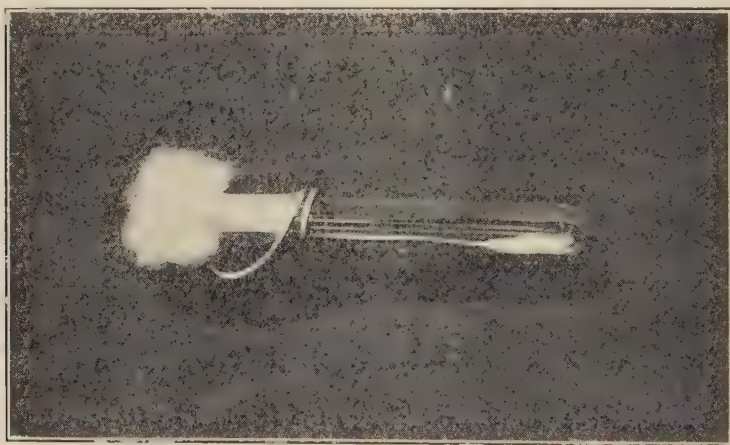


Figure 4.—The Wire Swab.

As the demands of the laboratory grew, it was found impossible to nick the wire by hand. A machine was designed which cuts the wire any length desired and nicks as it cuts. (Figure 5.) With this simple device and a little experience, laboratory workers can cut 1000 per hour. The machine will cut and nick approximately 100,000 wires before it has to be resharpened. We use a 13 gauge soft aluminum wire. It is cut and tied in bundles of 100 and sent out to a housewife to roll the swabs at 22 cents a 100. With the price of wire at 41 cents per pound, and the present labor charge in cutting wire, we are able to make these swabs for \$3.06 per 1000. The wire swab is not only economical but it adds to the accuracy of our cultural methods as the wire does not absorb moisture from the swab.

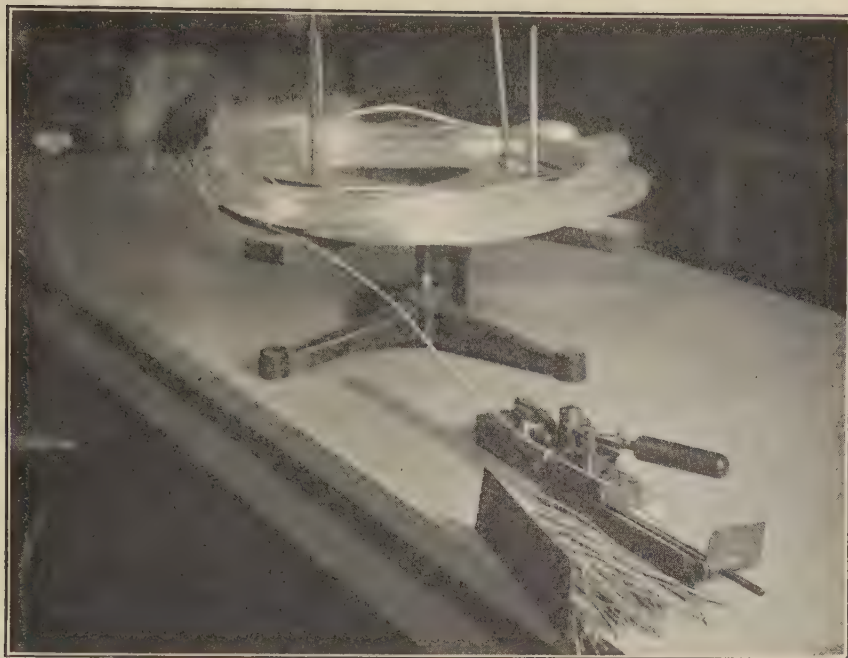


Figure 5.—Wire-Cutting Machine.

Modified Keidel Tube for Collecting Blood Specimens*

C. C. Young, Lansing, Mich.

Our modification of the ordinary vacuum tube which was devised by Keidel for collecting blood specimens, and which has demonstrated its usefulness in the hands of the practicing physician, will undoubtedly receive enthusiastic support from the laboratory technicians. From the laboratorian's point of view, the old form of Keidel tube is a nuisance for the reason that:

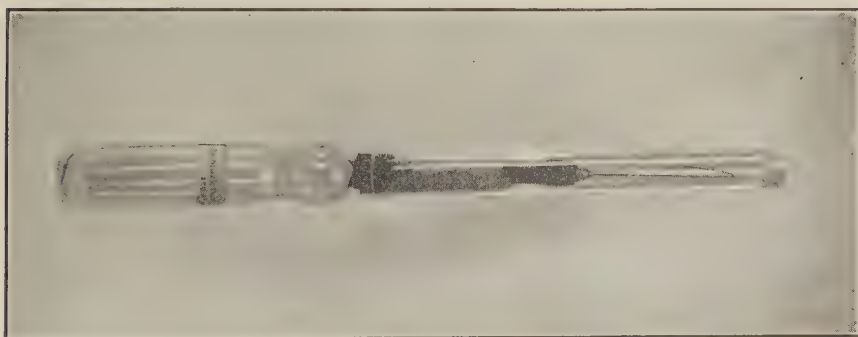


Figure 6.—Modified Keidel Tube for Collecting Blood Specimens.

* Reprinted from the Journal of the American Medical Association, 79:739 (Aug. 26) 1922.

1. It does not fit the standard centrifuge tubes of the International Centrifuge Company.

2. It is very difficult to gain access to its contents without risk of contamination.

3. There is constant danger of accidental cuts from jagged fragments of glass.

The design of the tube shown in the accompanying illustration is constricted and scored so that the top of the tube can be removed under aseptic conditions without danger to the operator. It holds approximately 8 cc. to the first shoulder, and fits both the long and the short types of brass centrifuge sleeves furnished by the International Equipment Company Centrifuge.

Until this type of vacuum tube is in use, we are inclined to discourage the use of the vacuum tubes by physicians, although we realize how much easier it is to collect blood in this manner than by the use of a sterile hypodermic syringe.

Specimen Containers

There are two sizes of specimen containers, one ounce and four ounces, closed with a Upressit cap which has a pure gum insert instead of a cork insert. These can be sterilized in the autoclave and the metal caps labelled with the department name. It was found that the paint which has a Prussian blue pigment turned brown in the autoclave, indicating that the jar had passed through the sterilizing process. The small jar is used for the collection of sputum, feces, blood for chemistry by the addition of sodium oxalate pellets, catheterized specimens of urine, spinal fluids, in fact any laboratory specimen, the rubber disc forming a satisfactory closure. There has been no difficulty experienced with contaminated specimens. Formerly when corks were used, it was found practically impossible to render them sterile. The large bottle closes with a Upressit cap and is used for water and urine specimens and found to be perfectly satisfactory.



Figure 7.—Specimen Containers.

In addition to these containers we use a two dram straight shouldered homeopathic vial with a No. 4 circle A cork for the collection of specimens for the serological examinations in syphilis. With these three containers we have been able to cut our costs a great deal.



Figure 8. Mailing Cases.

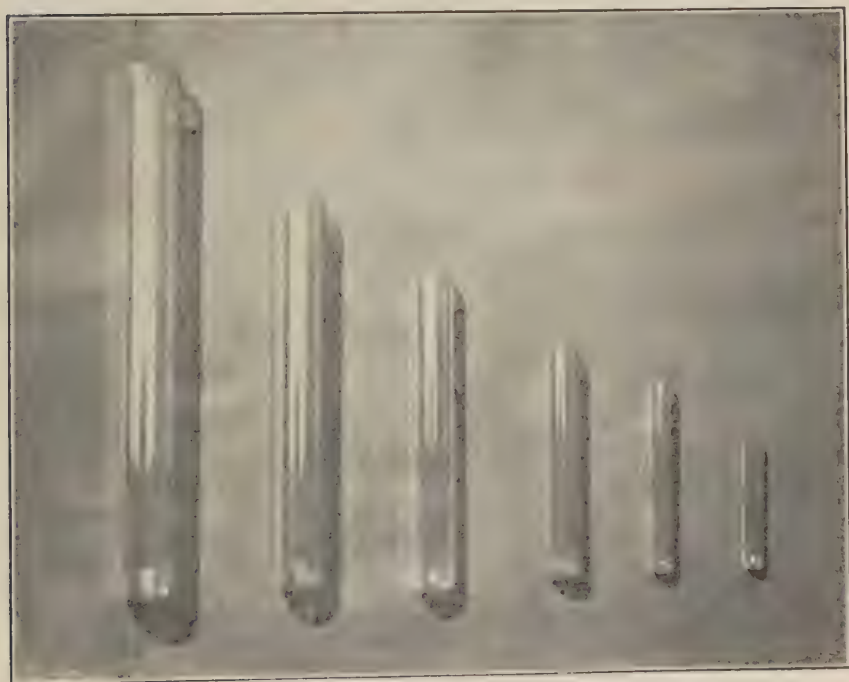


Figure 9.—Test Tubes for Routine Bacteriology and Serology.

Cotton

The Maplewood Mills at Fall River, Massachusetts, is our source of plugging cotton. The cotton used in the Michigan Department of Health laboratories is a special long fibre South Sea Island Cotton, graded to the trade number 1W and can be secured only in 10 pound header rolls.



Figure 10.—Standard Pipettes.

Test Tubes for Routine Bacteriology and Serology

Market conditions for test tubes have been such that we have been forced to write our own specifications as to quality of glass and dimensions of tubes. The confusion arising from various dimensions which would not fit test tube racks, slant racks, baskets, etc., forced us to standardize. We are using six sizes of tubes made out of high grade resistant glass which must contain no free alkali, judged from the following test. The test tube is filled $\frac{3}{4}$ full of distilled water, three drops of phenolphthalein indicator added. They are then autoclaved 15 pounds for 15 minutes. The solution must not turn pink.

Sizes of tubes

Major Use

Inside dimensions

2 x $\frac{1}{4}$

3 x $\frac{3}{8}$

3 $\frac{1}{2}$ x $\frac{1}{2}$

5 x $\frac{5}{8}$

6 x $\frac{3}{4}$

8 x 1

For inverted vial type of fermentation tube.
Wassermann and Kahn Precipitation Tests.
Agglutination tests.
Loeffler's medium and throat swabs.
Bacteriological cultures.
Durham fermentation tubes.
Special Work.

Mailing Cases

The mailing cases used by this department are furnished either by Improved Mailing Case Company, New York City, or the Ohio Paper Products Company, Massillon, Ohio.

Standard Pipettes

As the laboratory developed, a source of supply of uniform pipettes became of paramount importance. No two orders would be the same length or the same type of graduation and in many instances would not fit the copper cases we had in use for sterilization. We therefore designed a series of nine pipettes with the principal graduations entirely encircling the barrel, as shown in the illustration. (Figure 8.)

The three pipettes illustrated to the left are 9, 10 and 1 mil pipettes with single graduations for use in water and milk analysis. The second series of three, a 10 mil graduated in tenths, a 2 mil graduated in tenths and a 2 mil graduated in hundredths, are for use in both the bacteriological and serological divisions and therefore some are marked with "W" and some with "B" to facilitate sorting in the wash room so that they can be returned to the proper division without record keeping. The third series of three are a 1 mil graduated in tenths, a mil graduated in tenths to deliver to the point and a 2/10 mil graduated in thousandths to deliver to the point. These three are used almost exclusively in the serological division, so they are marked with "W" to facilitate sorting. A few are marked with "B" so that there are always a few 1 mil pipettes which would deliver to the point in the bacteriological division for agglutinations.

Culture Tube Baskets

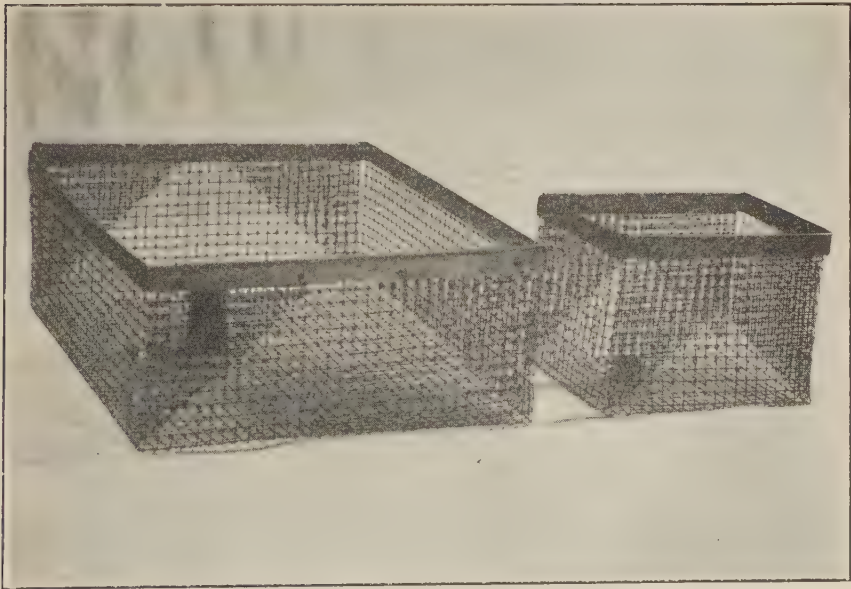


Figure 11.—Culture Tube Baskets.

The illustration shows two culture baskets, one 4 x 5 x 4 which holds 50 $3\frac{1}{2} \times \frac{1}{2}$ tubes and the other 8 x 10 x 4 which is a general stock basket of the laboratory. These baskets are simple in design. A rectangular piece of galvanized wire screening, $\frac{1}{4}$ inch

mesh, is shaped over a wooden block and the corners turned with a mallet. A tinned sheet metal binding is put on with one rivet, broken into place and fastened with a nail set, each punch mark passing through both thicknesses of the binding. The distinct advantage of this type of basket is that there are no soldered joints to weep in dry air sterilizer. Their cheapness recommends them to any large organization.

Shaking Machine

The development of the Kahn Precipitation test necessitated a shaking machine which would shake several racks of tubes at a time. The sliding rack holder is connected by means of a crank to the wooden pulley to an ordinary 110 volt alternating current motor running 1650 revolutions per minute. This is the cheapest type of motor and the shaking machine was built in our own instrument shop at a total cost of \$11.00 exclusive of the motor. There has been an enormous saving of time as it shakes six racks at a time at a uniform speed.

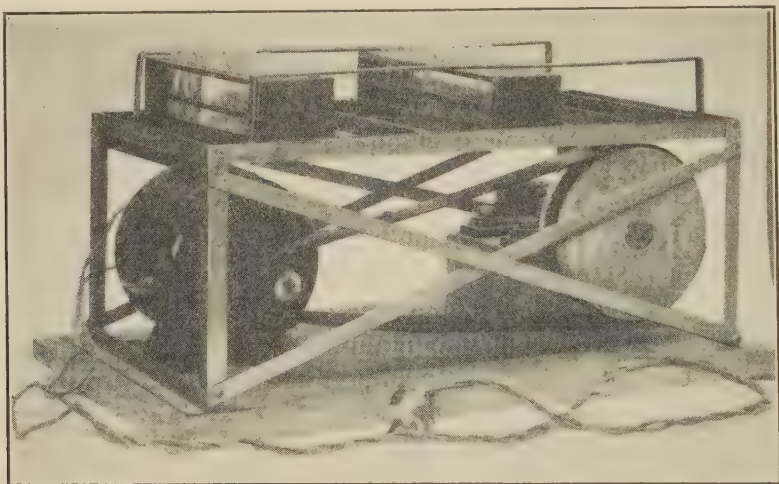


Figure 12.—Shaking Machine.

The test tube racks shown on the shaking machine are of tin lined sheet copper, made in our instrument shop, and carry three rows of ten holes, the center row being staggered so that the tubes in the center row are between the two outside rows. The dimensions of these racks are $2\frac{5}{8} \times 3 \times 11\frac{1}{4}$ and they cut very nicely out of stock sizes of sheet copper.

Standard Tubes and Pipettes for the Kahn Test

- a. Test tube used in carrying out precipitation tests. Inner diameter 0.9 cm. and length about 7.5 cm.
- b. Test tube employed in the preparation of standard antigen dilution. Inner diameter 1.5 cm. and length 5.5 cm.
- c. Pipette, 1 c. c. marked in 0.01 c. c.
- d. Pipette, 0.2 c. c. marked in 0.001 c. c.

Bleeding Outfit

An outfit designed to bleed and collect blood from a sheep under aseptic conditions. "A" represents the mouth piece which is plugged with cotton. The flask contains beads "B" for defibrinating blood. The outfit is sterilized in the autoclave.

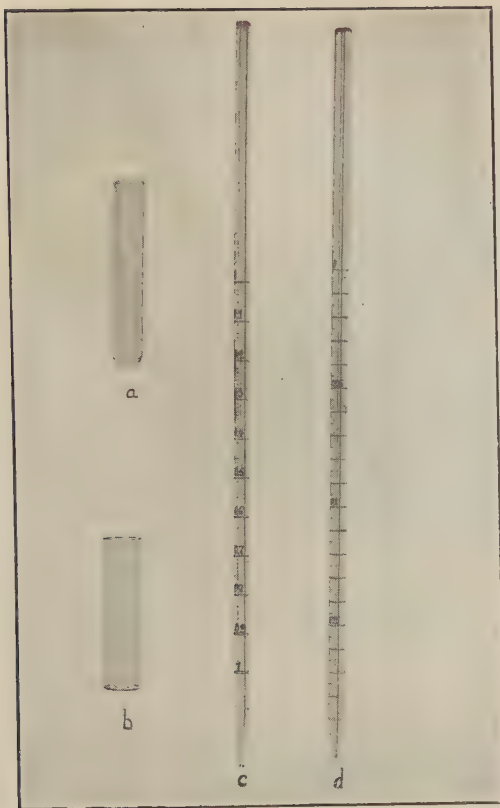


Figure 13.—Standard Tubes and Pipettes for the Kahn Test.



Figure 14.—Bleeding Outfit.

Agar Filtration Outfit*

Pearl L. Kendrick

All laboratory workers are familiar with the difficulties accompanying the usual method of filtering agar through cotton in a funnel. In order to avoid these difficulties, some laboratories, notably those of New York State, used a filtration method based on the principle of suction. Such a method is employed in the Michigan Department of Health laboratories. The accompanying illustration (Figure 13) shows the type of container and bottles used in these laboratories.

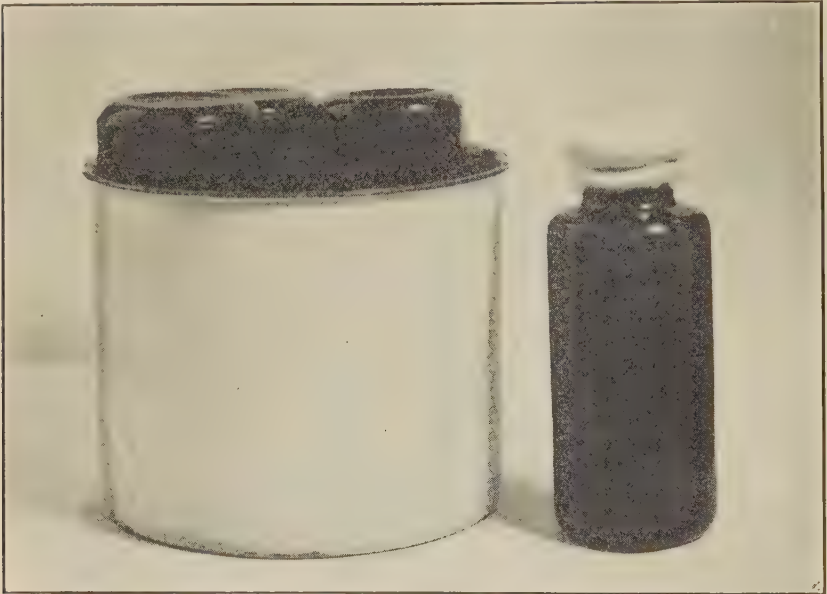


Figure 15.—Agar Filtration Outfit.

The empty, covered bottles are inverted in the container of melted agar medium, the container placed in a steam sterilizer (Arnold) and, in the case of 1.5 or 2 per cent agar, left for 30 minutes after the temperature is 98° C. to 100° C. The container is then removed from the Arnold and the contents left undisturbed at room temperature for the filtration to take place. The filtration of the agar into the bottles is usually complete in five to ten minutes after removal from Arnold.

Media Storage

Practically all media is stored in French Square bottles especially moulded with extra heavy walls, out of flint glass. These are also used for culture flasks in the preparation of vaccines or bacterial antigens.

* Abstracted from the American Journal of Public Health, 14:1077, Dec. 1925.



Figure 16.—French Squares for Media Storage.

A New Petri Dish Cover*

C. C. Young, Lawrence, Kan.

The ordinary porous Petri dish top has not given satisfaction in our water laboratory. In an attempt to eliminate the troubles a glaze was put on the outside of a few covers for experimental purposes. We were so pleased with the improved results obtained

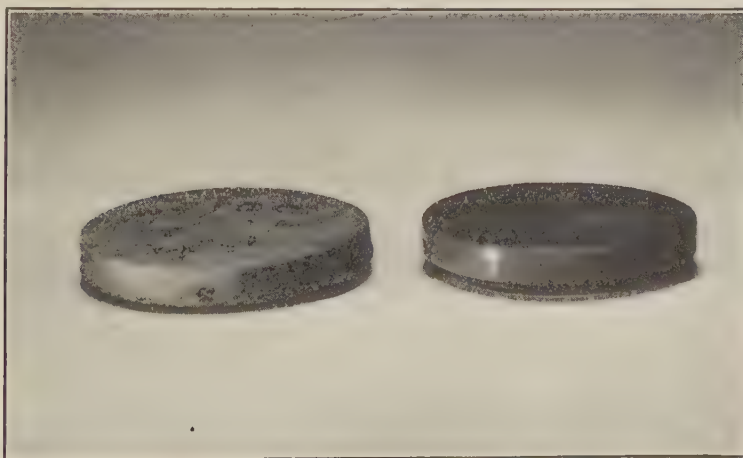


Figure 17.—A New Petri Dish Cover.

* Reprinted from the Journal of the American Medical Association, 63:1031 (Sept. 19) 1914.

that hereafter porous tops with glazed outer surface will be used entirely in this laboratory.

These glazed covers combine the good features of both the glass and the ordinary porous tops. They are superior to the unglazed tops in many ways and the cost is no greater.

1. Evaporation of the water from the medium is reduced about 60 per cent, but they are still porous enough to absorb all water of condensation.

2. The glass bottoms are not scratched and ground when the plates are stacked in the incubator.

3. The glazed surface affords a convenient place for marking with wax pencil. The marks can be rubbed off as easily as they can from glass.

4. The glaze adds much strength to the cover; consequently the breakage is reduced to a minimum.

5. They slip one over the other as easily as glass.

6. There is less danger of contamination, as the glaze protects the porous part of the cover so that material spilled on a plate is not absorbed.

Colored Plugs for Sugar Media

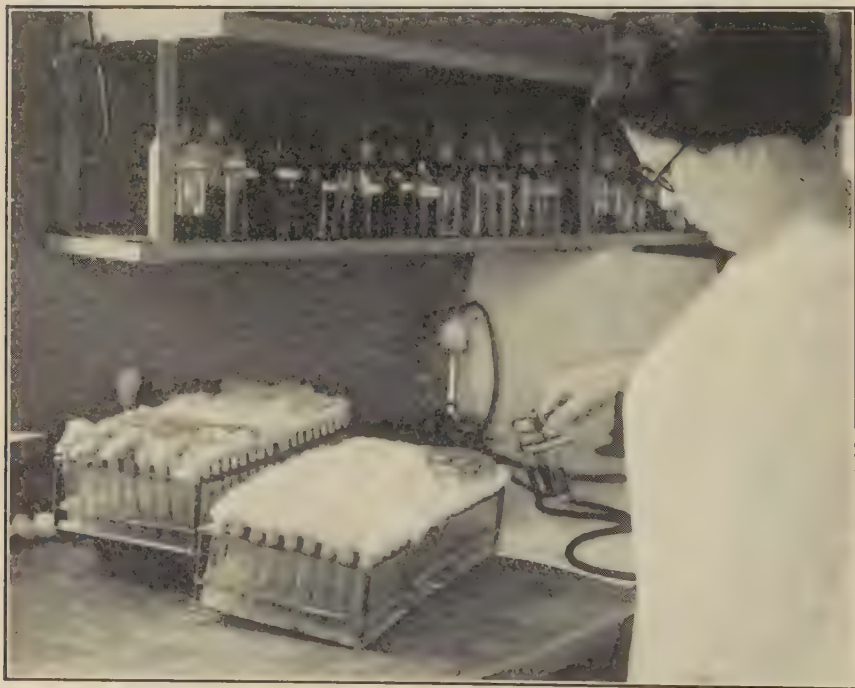


Figure 18.—Atomizer and Method of Coloring Cotton Plugs.

Some years ago, while at the Lawrence Experiment Station, Lawrence, Massachusetts, Mr. Stephen DeMerrit Gage showed how he marked his different sugar mediums by spraying a dye on the cotton plugs. Several times during the past fifteen years, the writer has tried to adopt this procedure. It always fell into disuse on account of the difficulty of keeping the atomizers in order. The simple DeVilbiss

compressed air lettering paint brush with changeable color cups solved the difficulty. Stock solutions of colors controlled by a key are kept in bottles. Thus a red cotton plug anywhere in our laboratories indicates dextrose media:

Dextrose.....red	Lactose.....blue
Mannite.....brown	Salacin.....purple
Inulin.....green	Xylose.....black
Sucrose.....yellow	Maltose.....salmon pink

Slant Racks

The slant rack shown in Figure 17 is made from three sheets of bronze 8" x 16" x 1/16" which is stock size. Two of the sheets are perforated with 9 rows of 18 11/16" holes with an eighth inch margin on one side and a half inch margin on the other. By reversing the perforated plates so that the narrower margin is superimposed upon the wide margin, then separating the sheets with brass tubing and bolting them together with 1/2-inch brass bolts, we have a rack that will hold 162 tubes, all with a uniform slant. With six of these racks in the autoclave we are able to slant 972 Loeffler's blood serum tubes at one time. The perforated bronze sheets can be secured from the Rochester Sheet Metal Works, Rochester, New York.

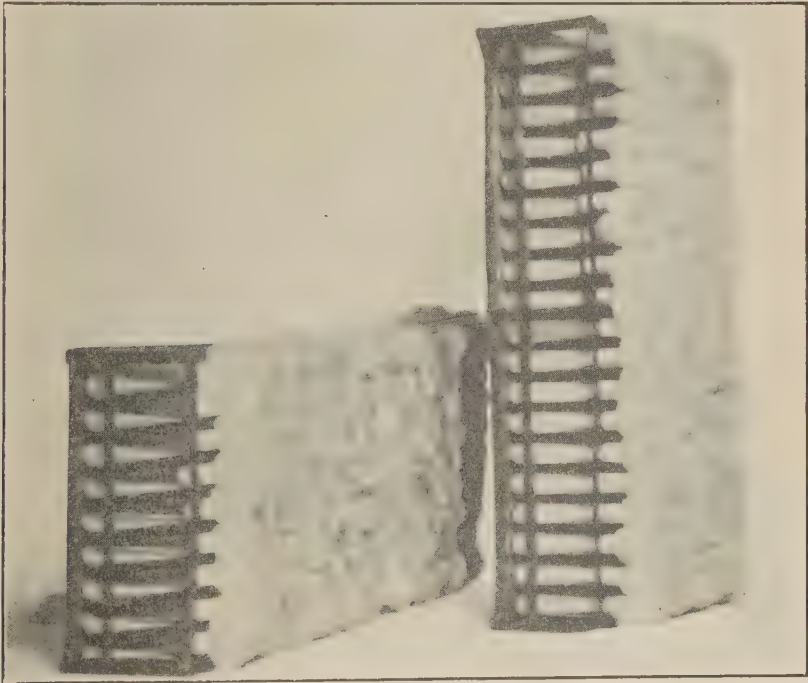


Figure 19.—Copper Slant Racks.

Incubator Exterior



Figure 20.—Incubator Exterior

Incubator Interior



Figure 21.—Incubator Interior.

E—INSTRUMENT SHOP

For many years the jobber's apparatus catalogs have carried a line of ovens and laboratory equipment made by the Apparatus Specialties Company of Lansing, Michigan. This company was owned, controlled and operated by Mr. Perry Edmonds. In 1922, he retired from active business life and left his patents with his assistant, Mr. Ralph Burd, B. S., who had been trained in his employ for many years. Mr. Burd found the business could not make a profit sufficient to warrant the investment of new capital in the enterprise. In 1922, he made a proposition to the director of laboratories which was acted upon favorably by the Commissioner and presented to the State Administrative Board for action and as a result the entire property of the Apparatus and Specialties Company was taken over by the State with the idea that there would be enough new instruments needed and enough repair work to do so that Mr. Burd could earn a good salary. In addition to maintenance of the equipment of the laboratory of the Michigan Department of Health and the laboratory of the State Department of Agriculture, Mr. Burd does the repair and instrument work for the Agricultural College, the state normal schools and the Superintendent of Capitol. Recently, the maintenance of all typewriters in the Capitol has been turned over to this new venture and another mechanic added to the staff. It was found that the cleaning and repair of the typewriters for the capitol cost the State of Michigan between \$6,000 and \$8,000 a year. Experience for the three months ending December 31, 1923, indicates that this work can be done for less than \$2,000 a year.

The saving of time and the increased operating efficiency to the equipment of the laboratory can only be appreciated by those fortunate persons who have had an instrument maker available. The writer can think of times when he has waited for six weeks for repair of cross-hairs on the eyepiece of a spectroscope when he should have made a report on an examination in 24 hours, and times when he has had to depend upon local electricians to fix a motor on a vacuum pump.

Altogether this instrument shop has been a very satisfactory self-supporting addition to the laboratory of the Michigan Department of Health.

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F—LABORATORY BLANKS

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